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THE ENERGY

OF

LIVING PROTOPLASM.

BY

Oscar Loew, Ph. D.,

Professor in the Imperial University, Japan.

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If there is one thing clear about the progress of modern science, it is the tendency to reduce all scientific problems, except those which are purely mathematical, to questions of molecular physics—that is to say, to the attractions, repulsions, motions, and co-ordination of the ultimate particles of matter.

T. H. Huxley, The Scientific Aspects of Positivism.

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PREFACE.

The interest which I have taken in the chemistry of proteids and in the chemical nature of protoplasm ever since I devoted myself to chemistry awakened the desire some years ago to frame some conception of the mode of origin of these substances in plants. Setting out from certain observations which bear upon the subject I became convinced that there must exist an unstable modification of albumin with which alone we have to do in living protoplasm and differing from that of dead protoplasm or from ordinary albumin, though easily passing into it. On this assumption I ultimately found myself able in great measure to account for the production of albumin itself as well as to enunciate laws of toxic actions which not only covered the facts already known but also others revealed by observations carried out to test their validity. Moreover, that hypothesis led *Dr. Bokorny* and myself to the actual discovery of a very easily changeable albumin contained as reserve material in various plants and remarkable for its properties.

The theory having thus proved its value as a guide to the discovery of new truth, I believe it will be useful to

give a survey of it and the facts on which it rests, descriptions of which now lie scattered through various publications. I trust that scientific readers will treat the question with unbiassed mind; all I ask for is impartial consideration and positive criticism.

Some of the chapters of this treatise have, in more or less abridged form, been communications to the *Bulletin of the Agricultural College of the Imperial University, Japan*, and in part have made the substance of lectures to scientific Societies in Tōkyō.

My special thanks are due to *Dr. Edward Divers, F. R. S.*, Professor in the Imperial University, for valuable suggestions.

Tōkyō, May, 1896.

Oscar Loew.

CHAPTER I.

VIEWS ON THE CAUSE OF VITAL PHENOMENA.

The change from life to death has always been considered as an insoluble mystery. The sudden stop of all manifestations of life, the irrecoverable loss of motion and sensation, appeared as melancholy as it was inconceivable. Even the gradual arrest of movement in infusoria or diatoms killed under the cover-glass while viewed with powerful microscopes, or the contraction of the protoplasm of the dying cells of *Spirogyra* when a deadly pressure upon the cover-glass is exerted, cannot fail to awake emotion and I confess this has made a more lasting impression upon my mind than the killing of an animal for culinary purposes. In what, now, consists the power, that produces the actions of life? In what consists this change to the rest of death? Before we enter upon our reply let us first review the opinions of the old philosophers and the modern physiologists.

Anaxagoras defined an animal as an automatic machine, but left undecided how it moved. *Socrates* ridiculed this idea, even on his death-bed.[1] According to *Aristotle* animal motions and animal heat are intimately connected, and the latter is produced by the food. The heart is the centre of motion and sensation, has a life of its own, and is the hottest part of the body.[2] *Plato* considered the red colour

(1) *Plato*, Phaedon 97, 98.

(2) *Aristotle* (Editio Bekkeri) de part. anim. II, 1; III. IV, 4 7; V. 2.—Hist. anim. I. XVII.—De respiratione VI.

of the blood to be an effect of the living fire, and the blood itself, as the bearer of the vital power, to be the seat of the soul.[1] The *Pythagoreans* defined animal life as the result of the entrance of the "life-spirit" into the body[2] by the respiration-process, and declared the brain to be the seat of sensation.

After the development of science was brought to a stand-still by various causes, biological questions took a new start in the seventeenth century. It was *Descartes*, who, in the year 1637, declared all powers of nature to be molecular motions; animals were in his opinion caloric automatic machines, in which the motions of the blood and of the organs were the effects of chemical heat-producing processes.[3]

With the observation of *Galvani*, in the year 1780, of the convulsions of a frog's leg brought in contact with two metals, indicating an electrical current, the view of *Galvani* found numerous followers, and men like *Humboldt* were among the admirers of the new vital theory. However, *Volta's* experiments on contact-electricity soon eclipsed *Galvani's* results and when the latter succeeded, in the year 1793, in adducing irrefragable proof of the presence of an electrical current in the animal, electrical contraction *without* metals, it did not find grace with the scientific

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(1) *Plato*, Tim. 493 etc.

(2) *Democritus*, in *Aristotle*, De respiratione, IV.

(3) *René Descartes* works, edited by *H. Kirchmann*, II. Soon afterwards (1687) *Mayow*, a now celebrated English *savant*, showed (*Opera omnia*) that life and fire are sustained by one and the same principle contained in the air, and that this principle is mixed with another indifferent substance. He can be considered not only as a forerunner of *Lavoisier*, but also as the first propounder of the doctrine of the conservation of energy.

public; the doubts and distrusts created by *Volta* were mightier than the language of the new experiments; *Galvani* got no satisfaction and died with the value of his work unrecognised. Nor when *Du Bois Reymond* proved, in the year 1843, the correctness of *Galvani's* views of the presence of electrical currents in animals, was one voice raised in support of the theory that electricity is the *primum movens* of all vital phenomena. Indeed, the electrical phenomena observed are, just like animal heat, but secondary actions, and not the first cause of life.

Another view gained much foothold also, one that was even defended by *Liebig*, the theory that organisms are ruled by a specific power quite different from any other, inscrutable to men, and supernatural: it was called *vital force.*[1] *Justus Liebig* said: "the cause of vital force is not chemical force, not electricity, not magnetism; it is a power that possesses the most general properties of all causes of motion, of variation of form and qualities of matter, and is a *specific* one, because it produces effects like those of no other power." "The laws of life, and every thing disturbing, promoting, or varying them, can doubtless be investigated, but without ever knowing what life really is."[2] Even in the third edition of his work, published in 1846, we find upon the first

(1) Attempts to define the limits of scientific inquiry in this direction have often been made in former and in recent years.

(2) Die organ. Chemie in ihrer Anwendung auf Physiologie und Pathologie, *Braunschweig* 1842, pp. 7 and 237. These few quotations will suffice to correct the erroneous statement, made sometimes, that *Liebig* was the first who combated the hypothesis of a specific vital power. Chap. 23 of *Liebig's* "Chemische Briefe" (1858) will be found especially instructive in this regard, as it exhibits *Liebig* as a most energetic defender of that hypothesis. The term *force* was formerly used in a more general sense than now.

page: "in the animal egg, in the plant-seed, is recognisable a remarkable activity, a cause of increase in substance, a compensation of loss, a power in the state of rest..... this power we call *vital* force." From page 225 the following characteristic passage may be quoted: "another fundamental error entertained by physiologists is, that physical or chemical forces alone or in combination with anatomy can suffice to explain vital phenomena."

Liebig did not share all the expectations created by the first synthesis of an organic compound, that of urea by *Wœhler* in the year 1828. Previous to this discovery it was often asserted that organic compounds could only be produced by "vital powers" and even *Berzelius*, but one year before, had expressed this opinion. This author, even in 1847, still declared his conviction that life is something inscrutable: "once connected with matter it produces development and growth, but how this proceeds is an insoluble mystery.[1] That life is something independent of matter but merely working through it, is even to-day a very general opinion.[2]

Mulder[3] believed that the specific vital power is intimately connected with the four elements found principally in organic compounds: carbon, hydrogen, nitrogen, and oxygen. *Hanstein* assumed the existence of a special psychic principle acting in union with physical and chemical power[4]; and *Bütschli* the presence of a vital ferment, localised in the nucleoli.[5]

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(1) Text-book of Chemistry, 1827, III, p. 1. Lehrb. d. Chem., 5th ed. vol. 4 p. 5.

(2) Cf. *Science*, March, 1893.

(3) Versuch einer allgem. physiolog. Chem. (1843).

(4) Das Protoplasma, p. 293 (1880).

(5) Zoolog. Anzeiger, 1882, p. 66.

Views very different from those of *Liebig* and *Berzelius* were entertained even a century ago by the German physiologist, *Reil*,[1] who wrote: "the so called vital force has fooled us long enough, and has led us into sterile deserts. Matter itself and not a specific new force is the cause of vital phenomena." In still stronger terms *Schleiden*[2] wrote: "Only ignorance and indolence of spirit are the defenders of the vital force in the present state of development of the natural sciences; of a power that can accomplish everything and of which nobody can tell how it acts or what laws it obeys. The savage, who takes a locomotive for a wild animal, is not more ignorant than the natural philosopher who talks about vital force in organisms."

In a similar sense, *Matteucci* declared that: "*parlare di forze vitale, darne la definizione, interpretare fenomeni col loro soccorso, e intanto ignorare le leggi di queste forze supposte, è dir nulla o è peggio che dir nulla, è appagare lo spirito, cessare dalla ricerca della verità.*"[3]

The opinion, expressed by the physiologist *Lehmann*, (1853) are of the same tendency; "though many vital phenomena are for the present inexplicable, we do not feel the necessity of assuming such a ruler as vital force."[4] Less hopeful of our ability to dispense with the aid of some such agency *Gorup-Besanez* (1874) said: "All the physical and chemical laws known to us at the present day are insufficient to explain the formation of a plant-cell, the process of generation, or the conduction of sense-impressions to the brain."[5]

(1) *Reils* Archiv, III, 424 (1798).
(2) Grundzüge der wissenschaftlichen Botanik I, 60 (1844).
(3) Lezioni sui fenomeni fisico-chimici dei corpi viventi., 2d. ed., p. 10 (1846).
(4) Lehrbuch der physiolog. Chem., 2d. ed., vol. III, p. 154.
(5) Lehrbuch der organ. Chem., 3d. ed., p. 6.

Haeckel,[1] however, finds the formation of a cell as simple as that of a crystal. He assumes a kind of "soul" in every atom and a progressive development of it in the organism.

Moleschott[2] defined life "not as the result of a specific force but as a certain condition of matter caused by peculiar motions produced by heat and light, water and air, electricity and mechanical actions," and *Heidenhain*[3] as a "peculiar connection of physical and chemical energies," which is essentially also the opinion of *Nencki*,[4] *Pflüger*, *Hüppe*,[5] *Halliburton*,[6] *R. Meyer*, *Helmholtz* and *Huxley*.[7] The last mentioned asks in a significant manner: "is the matter of life composed of ordinary matter differing from it only in the manner in which its atoms are aggregated?" *Cohn*[8] infers that the energy of the living organism must be of a mechanical order though as yet undecomposable into other known energies, while *Balfour Stewart*[9] writes: "In fine, we have not succeeded in solving the problem as to the true nature of

(1) Generelle Morphologie der Organismen, I, pp. 143 u. 148.—Cf. also Tageblatt der Naturforscherversammlung zu *München*, 1877, p. 22.

(2) Kraft und Stoff, 3rd ed., p. 256 (1856).

(3) Handbuch der Physiologie, V, p. 11.

(4) Arch. f. exp. Path. u. Pharmacol., 20, 343.

(5) On the Cause of Fermentations, etc., Berlin, 1893, p. 14.

(6) Chemical Physiology, Chapt. 14 (1890).

(7) Lessons on Elementary Physiology (1870), lesson VII, and in "The physical basis of life."

(8) Lebensfragen, Bot. Centralbl., 1886.

(9) Conservation of Energy, Chap. V (1875). In full accordance with *Balfour Stewart*, another prominent English *savant*, *H.E. Armstrong*, declared (Anniversary Address; Chemical Society, *London*, 1895): "Whatever the nature of the protoplasmic molecules they must be of extreme complexity and, consequently they must present very many *active regions* to which other groups may become attached and within which therefore circuits can be established; hence the marvellous power of protoplasm of conditioning a great variety of changes; and it is doubtless the marvellous complexity of the albuminoids which renders such diversity of type possible in both the animal and vegetable kingdoms."

life, but have only driven the difficulty into a borderland of thick darkness, into which the light of knowledge has not yet been able to penetrate." "We have now to remark that the particular force which is thus used by living beings is chemical affinity. Our bodies are, in truth, examples of an unstable arrangement of chemical forces, and the materials which compose them, if not liable to sudden explosion, like fulminating powder, are yet pre-eminently the subjects of decay."

Also *Tyndall* (1874) confessed[1] that he discerned in matter "the promise and potency of every form and quality of life." In doing so, as has been so acutely pointed out by *Stallo*,[2] he only then re-worded an old thought of *Francis Bacon*: "And matter, whatever it is, must be held to be so adorned, furnished, and formed, that all virtue, essence, action, and motion may be the natural consequence and emanation thereof."[3] The conviction that *chemical science* will principally be concerned in the solution of vital problems is emphatically expressed by *Erlenmeyer's* appeal: "if physiologists like *C. Ludwig* identify the progress of physiology with the progress of chemistry, then the chemists must feel anew instigated to devote themselves to physiological problems."[4] And not less decided is the opinion of *Berthelot* on this point, when he declared his object to be '*ramener la chimie tout entière* . . . au mêmes principes mécaniques qui régissent déjà les diverses branches de la physique.'

The collection of views, hypotheses, and declarations we

(1) Inaugural Address to the British Association at its *Belfast* Meeting.
(2) Concepts and Theories of Modern Physics, *New York*, 1884.
(3) De Princ. atque Origg., Opp. ed. Bohn., 2, 691.
(4) Zeitschr. f. Chem., 1859.

have ventured to present to the reader will certainly prove of some interest. One author merely denies current ideas; another presumes, or asks questions; a third makes positive statements without any proof whatsoever. Nowhere do we find the least attempt to attack the questions at issue by experiment; everywhere it becomes evident, that in using the word *life* the authors have not distinguished properly between the *Energy* of living matter and vital *Functions* which stand to each other about in the same relation as the pressure of the steam to the working of an engine. It is one question what energy is the *primum movens*, but there are others much more complicated as to how this energy is utilised to perform various functions and how the machinery is constructed. Just as steam can be used for the most different performances, so vital *energy* serves for a variety of vital *functions*. And the comprehension of these functions, again, can be attained only by commencing with the simplest forms of life, as *Claude Bernard*[1] has justly pointed out: "*Pour comprendre les fonctions de l'organisme, il faut connaître celle de la cellule.*"

(1) *Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux*, pag. 458.

CHAPTER II.

CHARACTERISTICS OF PROTOPLASM.

The discovery of the morphological units of complex organisms are of comparatively recent date. But although many investigators have contributed their share towards creating a stock of anatomical and physiological knowledge and a great number of phenomena have been described with minute exactness, final satisfactory explanations have not been reached.

After *Robert Hooke*, in the middle of the seventeenth century, had discovered the cellular nature of plants, *Malpighi* and *Grew* had closely investigated their anatomical structure, and *Corti*, in the year 1772, had observed the circulatory movements in plant cells, a long time elapsed before *Schwann* promulgated his cell theory in 1839. *Schleiden* had recognised about two years previously that each cell is the product of another and is never spontaneously formed in the fluids of the organism. He declared: *omnis cellula e cellula.* The cellular nucleus was first described by *R. Brown* in the year 1833, while the animal cytoplasm was discovered by *Dujardin*, who called it *sarcode.* *Mohl* observed later, in the year 1844, a similar substance in plant cells which he designated as *protoplasm*, whereupon *Max Schulze* demonstrated the close *chemical* analogy between the living matter of animals and plants. A *cell* was defined as nucleated protoplasm with or without a cell wall, the *nucleus* as a

network of filaments containing the nuclear fluid and covered by the nuclear membrane; the surrounding protoplasm, again, as a mixture of solid and liquid material, forming a tenaceous transparent mass of neutral or weak alkaline reaction, which was later designated as *cytoplasm* in contradistinction to the protoplasm of the nucleus and of the chlorophyll bodies.[1] Further microscopical investigations revealed the important rôle of the nucleus and of the centrospheres with the centrosomes in the multiplication of cells and in the sexual propagation of organisms.[2]

Gradually it became clear that protoplasm was an instrument of great complexity, especially in a germ cell, which are, to speak with *Maxwell*, "representative bodies containing members collected from every rank of the long drawn ramification of the ancestral tree, the numbers of these members being amply sufficient to furnish characteristics of every organ." Our microscopes, however, do not reveal the systematic arrangements of the smaller particles, and even the coarser structures, alveolar or reticular, are, as *P. Klemm* has shown, not fixed but changeable and often merely appearing at the moment of death; only the fibrillar structure of the nucleus in its living state seems to be fully established. At any rate it may be convenient to distinguish by 'tectonic' the *invisible* molecular arrangement in protoplasm, from the *visible* differentiation

(1) The further distinctions of polioplasm, filarplasm, hyaloplasm, archoplasm, kinoplasm, trophoplasm, and idioplasm may here be passed over.

(2) Many authors participated in the discoveries, I need mention only *Flemming, Strassburger, E. Zaccharias, Carnoy, Van Beneden, Boveri, Guignard.*

within cells aud multicellular organisms which is 'organisation.'

Innumerable cells of various forms and functions compose the organs of motion, secretion, sensation, and generation, and many sacrifice their individual existence in support of the whole. The multifarious actions and the combined efforts of various cell systems in a multicellular organism secure the proper condition for its existence. One of the most important properties of protoplasm coming here into play is *irritability*, which no organism can dispense with. In virtue of it even the lowest forms of life are capable of responding to external influences of exceedingly subtle nature.[1] With the highest development the processes known as sensations are connected with specialised organs, the nerves, in which accumulation, conduction, transformation, and translation of energy is carried on (*Rosenbach*).[2] Part of this remarkable system is the brain with myriads of connecting and ramifying threads and those cephalic ganglia denominated by *Huxley* 'registering apparatus.' *Huxley* defined a nerve as a 'linear tract of specially modified protoplasm between two points of an organism, one of which is able to affect the other by means of the communication so established,' and thought it conceivable that the lowest animals and even plants might have a kind of nervous system.[3]

(1) Thus the infusorium, *Paramœcium*, is sensitive for differences of temperature as small as 0·1° C. (*Mendelsohn*, 1895).

(2) The muscles also are highly irritable so that grazing with the tip of a pin will produce a contraction whereby an increased amount of oxygen is absorbed with liberation of heat and electricity (*Pflüger*).

(3) Lecture at the Royal Institution, London, 1876. Cf. also the interesting deductions of *F. Hüppe*, Naturwissenschaftliche Einführung in die Bacteriologie, pag. 136.

Most extraordinary appear the functions of the nerves of the higher animals; not only the finest differences in the waves of light and sound are perceived with great precision but also surprisingly small quantities of certain compounds can be noticed by the olfactory and gustatory organ.[1] As little as 0·0006 thousandths of a milligram of vanillin can, *e.g.*, still be noticed by the smell (*Passy*). Similar wonders, however, are presented by the vegetal protoplasm; of diammonium phosphate 0·0000033 milligram suffices to cause the inflexion of the tentacles of *Drosera* (*Darwin*). *Pfeffer* has noticed that a solution of 0·01 % of sodium malate can attract spermatozoids of ferns[2] and that movements of bacteria are detemined by the attraction or repulsion they experience in contact with certain substances (chemiotaxis). Again, the direction of the growth of the mycelium of fungi is guided by the presence of nutritive compounds (*Miyoshi*), and most minute quantities of certain still unknown substances (enzymes?) can produce various galls on leaves or start an abnormal growth in branches (hexenbesen).

Sensitiveness to sunlight is in plants not only manifested by heliotropism but also in various other ways; thus *Merulius lacrimans* as well as *Hydrodictyon* are induced

(1) It may, however, be mentioned here that compounds of the widely different chemical constitution can produce sometimes very similar impressions upon the olfactory or gustatory nerve. Thus, several nitro-compounds (nitroflavoline and trinitroisobutyltolyl ketone) possess the odour of musk; alkaloids as well as non-nitrogenous componuds may have nearly the same bitter taste and certain benzene derivatives (Remsen's saccharin; dulcin; amidocamphor) and dimethylurea taste as sweet as sugars.

(2) Maleic acid also attracts them, fumaric acid does not (*Pfeffer*). Of peculiar interest is also the difference between the behaviour of malic and that of the closely related tartaric acid upon the tentacles of *Drosera;* the former causes inflection, the latter does not (*Darwin.*)

to form spores (*R. Hartig*; *Klebs*). Irritability to molar energy is not only noticed in geotropism but also in the inflection of the tentacles of *Drosera* by a hair of 0·00082 milligram in weight (*Darwin*), or in the influence by a thread of 0·00025 milligr. upon the direction of a tendril of *Sicyos angulatus* (*Pfeffer*).[1]

Truly, problems of most intricate nature are involved in the differentiations of irritability and tectonic. One thing, however, is certain: before the first step towards solving the more difficult problems can be made it is absolutely necessary to answer the fundamental question: What is the cause of the never-ceasing cell-activity? What is the nature of the energy governing the living cell?

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(1) Hydrotropism and aërotropism are but special cases of chemiotropism. Also the stimulation of certain chemical activities by very small quantities of poisons belongs to this class of phenomena.—A case of thermotropism with a bacterium was observed by *Beyerink* and a case of negative electrotropism with *Phycomyces* by *Hegler*.

CHAPTER III.

PROTEIDS AND PROTOPLASM.

The fact that with the differentiation of the protoplasm distinct classes of cells are produced, that do continually a specific kind of work as long as they live, can only be attributed to a special and fixed system of tectonic, or structure, for each separate case. A change of construction, an alteration in the system, must interfere with the normal working and produce an entirely different result. A comparison with the multitude of machines used in industries, all built of the same material and moved by the same energy but doing very different work according to their construction, will here suggest itself to the mind. Any injury to an important connection will at once stop the normal working.

If we now consider protoplasm from the *chemical* point of view, the constant presence of *proteids*[1] must strike us as the most important feature. In a cell we can discern principally albumin and nuclein;[2] the former, along with some nucleo-albumin, in the cytoplasm, the latter as constituent of the nucleus and containing as chief component,

(1) The name *proteid* is used here in the general sense, not in the restricted meaning of some German authors.

(2) We leave out of consideration here the many kinds of *reserve* proteids. *Osborne* and *Vorhees* (7th Ann. Rep. of Connect. Agr. Exp. Stat., 1893) have shown the presence of six different reserve proteids in wheat-grain alone; *Osborne* also four in cotton seed, three in oats and flax seed. Cf. also the investigations of *Ritthausen* and others.

besides metaphosphoric acid, also albumin. Another proteid called plastin, less easily attacked by acids and alkalies than nuclein but related to it, was observed in plant cells by *Reinke* and by *Zaccharias*. Other compounds present may vary in quality and quantity, according to the species and state of nutrition or development of the organism; they may be either required for respiration and other useful purposes or they may be mere by-products; but *they cannot possibly be essential to the causation of the vital properties of protoplasm.* Besides the occasional absence of these compounds as an objection to their being essential in causing vital action, it has to be pointed out that there exist organoids of the vegetal cells, as, *e.g.* the tonoplast, that contain absolutely nothing else but proteids, water, and mineral matter. In the cytoplasm itself, however, are often found lecithin and cholesterin, fat globules and carbohydrates. But even though the quantity of these be sometimes larger than that of the proteids, that cannot possibly tell against the leading rôle of the latter.

Earlier authors defined as protoplasm the entire mixture of various compounds present[1]; others, led by *Pflüger* and *Hanstein*, reserved that name for the organised proteids only. How, however, in view of the constancy and uniformity of the work of a given protoplasm, the former view, that by the co-operation of all the compounds present the vital properties are produced, can still find defenders at the present day, remains incomprehensible to a logical mind. We are left by that view to wonder why in the dead cells that *mixtum compositum* which is still present does not ever, by some instigation or other begin to co-operate again and exhibit the activities of life once

(1) Compare, *e. g.*, *Reinke's* definition; Botan., Zeitg. 1883 p. 66.

more. Some one who found 27 per cent calcium carbonate in a fungus (*Æthalium*) even concludes that this belongs to the molecular system of the protoplasm of this organism! But if all the substances found in protoplasm were to be essential for its constitution, then all secretions, numerous organic acids, tannins, alkaloids, hydocarbons, esters, and wax, urea and uric acid in animals, etc. would all be parts of various kinds of protoplasm. All these substances are *formed* in the protoplasm of different cells and must exist there, even if only for a short time. This older view of declaring everything found in protoplasm to be an essential part of it and attributing to it even vital actions, must therefore lead simply to absurdities.

But a new definition, a new conception, will always be considered as a sort of challenge by those who cling to the old notions; no wonder then that in the attempts to defend the impossible very odd ideas are often expressed. Thus, we find in an attack upon the new proposition, among all sorts of objections and declarations that do not hit the points in question, the following passage: "Is it thinkable that the vital properties are connected with only one class of compounds? Why not ascribe them to *water* which in quantity exceeds that of the proteids, or why not to the lecithin or to the potassium salts which are of general occurrence in the cells?" This will suffice as an example of the conceptions still prevalent in the year 1882. Reflection must lead us logically to consider the proteids to be pabulum of the protoplasm. Indeed a protoplasm without proteids would be a non-entity, while it might contrariwise be asserted that one without any other organic compound but proteids could very well exist, in case reserve-proteids are stored up in it as food.

If, then, we must look upon the *proteids* as yielding the vital phenomena and observe that the chemical properties of dead and living cells are totally different, there remains no other conclusion but that the *proteids* of the *living* protoplasm undergo a *chemical change at the moment of death.* The fact that in the living cells a continuous combustion is going on, in the dead ones not, led *Pflüger,* even in the year 1875, to infer that the chemical nature of the proteids is altered.[1] Other chemical facts, however, lead to the same conclusion, such as that there are many *poisons* having no action whatever on *common* proteids, which act upon the proteids of the *living* protoplasm (cf. Chap. VI).

Pflüger also pointed out that the decomposition of albuminous matter in the living animal organism yields other products than the decompositions artificially accomplished in the laboratory, and concluded that the nitrogen

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(1) *Pflüg.* Arch., 10, 320. We may quote as illustration of the untenable position of the opponents of this view the following passages: "'Why should just the *proteids* and not the *water* undergo a chemical change when the cell dies?" was objected by one who evidently had forgotten the existence of isomerism. "That in living cells chemical processes go on which are no longer observed in dead cells, is an old observation, hence the assertion that the living cells are chemically different from the dead is nothing new," was objected by another adversary, who thus proved that he did not distinguish between a *fact* in itself and an *explanation* of the *causation* of the fact. Such have been the attempts to discredit the new doctrine.

As to the function of the water, nobody would deny its great importance. We see on the one hand organisms die that lose a certain amount of water and on the other hand the embryo of seeds awake to life when water has entered. But it is evident that water cannot be the "primum movens." The vital energy in a seed is a suppressed kinetic energy. The force of cohesion is here opposed to plasmic energy, but as the state of cohesion is changed by the entering water, the energy can be displayed, the *solicitation* is n

of the "living albumin" is linked to the other atoms in a different manner.[1] He supposed that it is linked as in the cyanogen-group, and that this group becomes changed by fixation of the elements of water when the cell dies:

$$.CN + H_2O = .CO.NH_2$$

This hypothesis, however, is not supported by chemical facts. Certain cyanogen compounds are, it is true, very unstable, being very easily converted into polymeric forms, and isocyanic acid is quickly decomposed by water into carbon dioxide and ammonia, but such cases cannot serve here for comparison; neither can the transformation of ammonium isocyanate into urea serve as an example; nor the transformation of nitriles into amides. And just as little probability exists for the hypothesis of *Latham* who assumes the existence of a series of cyanhydrins attached to benzene derivatives[2] and a chemical change of these groups at the moment of death. Nevertheless, it was evident that some chemical change, whatever it might be, must certainly take place in the proteids, although the physiologists were few who openly admitted this.

Among them stood conspicuous *M. Nencki*, who declared: I have repeatedly expressed my opinion, that investigation into the albuminous bodies must take a new direction, if we want to undertake with success the closer investigation of those actions we call "life;" the pro-

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longer prevented from taking effect, and the first sign of life makes its appearance in form of respiration. The dormant life of certain seeds can be preserved for over fifty years (*A. Peter*).

(1) This conclusion however he was not justified in drawing, for the same albuminous matter can yield under different conditions very different products. Besides, certain nitrogenous compounds of the animal are formed by *synthetical* operations, as uric acid or urea.

(2) British Med. Journ., I., 626 (1886).

teids of the living cells must have another chemical constitution than those of the dead cells." [1] And, again, "It is of essential significance, that albuminous substances, isolated at very low temperatures from living animals, are of a very changeable character, as, *e.g.*, blood-plasm, oxyhæmoglobin, and myosin." "The most important function, nay even the most characteristic feature of life itself, is the formation of labile albumin molecules."[2]

Rosenbach[3] and *Detmer*[4] may also be cited, the former having declared that "Death is the upset of the unstable equilibrium of atoms in the living matter," while the latter assumed *that all the atoms* of the "living albumin molecules" are in such a lively motion that a dissociation can easily take place forming thereby, on the one hand, non-nitrogenous compounds such as glucose and, on the other, amides. By this *dissociation* respiration would be induced and other vital phenomena made possible. Such a hypothesis, however, is incompatible with the great sensitiveness of the protoplasm towards every disturbing influence. The continuous *regeneration* supposed by *Detmer* would be wholly impossible, as dissociation would lead to death.

Now, if the view is correct that some chemical change does take place, it must further be recognised that when the dead matter of our food is converted into the *living* matter of our nerves, muscles, and glands, a considerable

(1) Arch. f. exper. Pathol. und Pharmacol., 20, p. 343 and Journ. f. prakt. Chem., vol. 26.

(2) Pflüg. Arch., 31, 336; Ber. D. Chem. Ges., 18, 385.

(3) Aufgaben der Therapie, Chap. 14 (1891).

(4) Vergleichende Physiologie des Keimungsprocesses. *Jena.* 1880. Ber. D. Bot. Ges., 1893; cf. also the views of *Beale.*

chemical change must also take place and just in the opposite direction to that connected with the loss of life. *Pflüger* (*l. c.*) expressed his conviction in the following words: "An albumin-molecule, which in the brain concurs in the production of thought, which in the spinal column mediates sensation, which in the muscles performs mechanical work, or in the glands starts chemical activity, is doubtless derived from the same dead albumin of the blood, but it is changed in chemical character as soon as it enters the living cell.[1] From the moment it forms a part of the living protoplasm, it commences to respire, to live. Only the *cells* have the property of life; such albumin, which has not become protoplasm, is dead albumin, even in the living body."[2]

As living cells are so easily killed by chemical treatment and an investigation as to the chemical state of the living protoplasm, carried on in the usual way, must be excluded, other means have to be resorted to (cf. Chap. VI).

The *ordinary* proteids, however, have often been the object of chemical investigation but the manifold observations

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(1) This production of living matter from dead was declared by *Pflüger* to be one of the greatest enigmas of nature (Die allgemeinen Lebenserscheinungen, Bonn 1889). *Pflüger's* supposition, that *Liebig* entertained the belief in a *chemical* difference between albuminous matter in living and dead cells, is an error. Nowhere in *Liebig's* writings can a decisive opinion be found.

(2) Later investigations however, have shown that the *dissolved* proteids of the animal body are by no means ordinary inactive proteids. *Rudolf Emmerich* was the first who observed, in 1887, that bacteria are killed by the blood of a living animal. The serum of dog's blood quickly kills leucocytes of man and rabbits. *H. Buchner* found that these properties of the dissolved albumin are lost at 55° C.

relating to their decomposition under different conditions are partly not suitable and partly still too imperfect to lead to a correct view as to the chemical structure of the proteids. However, a brief survey as to the principal chemical characteristics may here be in order.

Numerous analyses of albumin led *Lieberkühn* to the formula $C_{72}H_{112}N_{18}SO_{22}$ which expresses in the present state of science as nearly as possible the relation between the numbers of atoms but not yet the molecular magnitude, which in all probability is a multiple of this expression.[1] Certain proteids, *e.g.*, hæmoglobin, have a still higher molecular weight than albumin. While crude albumin is easily soluble, pure albumin, free of any trace of inorganic matter, is insoluble in water, but easily soluble in very dilute hydrochloric acid. Acid solutions are easily precipitated by neutral salts, while alkaline ones are unaffected by them. The general chemical character is that of amido-acids, *i.e.*, the proteids can play the part of weak acids as well as that of weak bases. Moreover, they are capable of passing into widely differing products, according to the nature of the chemical attacks made upon them, a behaviour which in all probability is due to a special facility of atomic *migration*, in consequence of the presence

(1) While *Lieberkühn's* formula might perhaps correspond to that of propeptone proper, the molecular weight of deutero albumose was found by *Sabanujeff* and *Alexandrow*, by the cryoscopic method, to be about 3200, or nearly double that which *Lieberkühn's* formula would lead to, and that of *albumin* to be 14270, *i.e.*, nearly nine times as high, while *Siöquist* recently calculated from different experiments 800 only. On the other hand, the molecular weight of *peptone* was found by *Paal* and by *Ciamician* to be much lower than supposed, *i.e.*, to be less than 400, and that of antipeptone or carnic acid by *Siegfried* to be 257. Peptone is, according to *Paal's* view, a mixture of several compounds.

of many hydroxyl groups, whereby affinities are loosened, as in the sugars. The sugars yield upon boiling with strong mineral acids products[1] which no chemist would dare to assume present as such in the molecules. An analogous case is exhibited by the proteids, which yield upon such decomposition amido-acids and bases.[2] The older view assumed the existence of these complexes in the proteids, supposed to be complicated ureas or guanidines in which the hydrogen atoms were replaced by the radicals of those amido-acids and bases.[3] Such a compound, however, would not be capable of yielding so great a variety of decomposition products, under different chemical or physiological influences. Thus, we observe the formation of pyrrol, indol, skatol, and methylmercaptan (*Nieber*), by fusion with caustic potassa; the formation of skatolacetic acid by certain microbes[4]; that of kynurenic acid, an oxyquinoline carboxylic acid, in the liver of the dog; that of glucose, glycogen, and milk-sugar in the body of carnivorous animals[5]; while probably

(1) Humic acid, furfurol, lævulinic acid, formic acid, and carbonic acid.

(2) Leucine, tyrosine, phenylamido-propionic acid, aspartic acid and glutamic acid, lysine, lysatine (*Drechsel*) and arginine (*Hedin*).

(3) We can conclude with a high degree of probability that the leucine radical does not exist as such in the proteids, not only from the behaviour towards potassium permanganate but also towards osmium tetroxide, which is reduced to metal by every compound containing the group $-CH_2-CH_2-$ and therefore by leucine, but not by albumin in the dark. It is true, many compounds show other reactions when liberated from complex molecules, but this is only the case when a reacting group becomes generated by that liberation, as, *e. g.*, in the hydrolysis of cane-sugar, whereby aldehyde groups are generated. Such objections have no weight in our case.

(4) *Nencki*, Wien. Akad. Ber., 1889.

(5) Some authors deny that any carbohydrate can be obtained from albumin; others, like *Pavy*, assert that it is possible by treatment with

also guanine, xanthine, and related bases are first produced from albumin before they become constituents of the nucleus.

Moderate oxidation of albumin by potassium permangante yields oxyproteosulphonic and then peroxyproteic acids,[1] while further oxidation leads to disruption of the molecule and formation of benzoic, succinic, acetic, formic, oxalic, hydrocyanic, and carbonic acids, besides ammonia and sulphuric acid.[2] There exist—to judge by the amount of bromine taken up by dry albumin—only two pairs of doubly linked carbon atoms[3] of the ordinary kind in the molecule, if *Lieberkühn's* formula is taken as foundation, while the existence of several benzene rings is highly probable and generally assumed.[4] As to the smaller atomic groups there are neither ketonic, and probably neither nitrile nor carboxylic groups present in the *ordinary* albumin ;[5] nitrogen is in all probability partly present as amido-groups,[6] partly as imido-groups ; and sul-

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bases to get sugar. *Schützenberger* mentioned long ago that a dextrin-like body can be obtained by their decomposition with barium hydroxide.

(1) *Maly*, Jahresber. f. Thierchem., vol. 15. This author found among the decomposition products of peroxyproteic acid by barium hydroxide a large amount of oxalic and glutamic acids.

(2) *Loew*, Journ. f. prakt. Chem., *31*, 153.

(3) *Loew*, ibid, pag. 139.

(4) *Nencki*, *l.c.*, assumes the existence of an oxyphenyl, a phenyl, and a skatol radical.

(5) On the behaviour of nitriles to hydroxylamine, cf. *Tiemann*, Ber. D. Chem. Ges., 1884, p. 128. If my hypothesis is correct (cf. the following chapter) then the carbon is present essentially in the form of CH, CH_2, and CHOH groups. *Lorenz* showed that the groups $O.CH_3$ or $O.C_2H_5$ are not present in albumin but are apparently so in small quantity in nucleins.

(6) Some difference as to the relative number of the amido-groups may exist in propeptone and albumin, since the former yields at once an insoluble compound upon addition of form aldehyde, the latter only after some time or on addition of acids.

phur as hydrosulphyl. Ordinary albumin, although easily yielding to the attack of stronger chemical agents, does not belong to the labile compounds in the proper sense of the word, which implies a much more subtle nature.[1]

It appeared to me that more insight into the chemical nature of the proteids could be obtained by the study of the *formation of proteids in plant cells.* Starting from a series of observations I reached a hypothesis, which led me to infer the existence of a labile and a stable modification of albumin, as explaining in a more satisfactory manner than the views of *Pflüger* and of *Latham* (see above) the difference between the proteids of the living protoplasm and those of the dead (cf. Chap. IV, V, and VI).

(1) The efforts made by some authors to reach proteids by synthesis would—leaving all other objections aside—never lead to such proteids as compose living protoplasm, since the methods employed would be wholly unsuitable for the purpose.

CHAPTER IV.

THEORY OF THE FORMATION OF ALBUMIN IN PLANT-CELLS.

The formation of albuminous matter in plant-cells is certainly one of the most important processes in the domain of general physiology, for upon it depends the growth of protoplasm, the multiplication of cells, the development of plants, and, through plants, the possibility of animal life.

This process, however, is not less interesting from a *chemical* point of view, as relatively simple substances are with astonishing facility converted by it into the most complex compounds known. Let us see whether certain principles of the nutrition of the lower fungi and of the phænogams will not furnish some clue to this extraordinary process.[1]

A. Nutrition of Microbes and Mould fungi.

Among fungi the *microbes* are especially remarkable for the intensity of their chemical activity. Oxidations and decompositions are accomplished in them on an extensive scale. Numerous compounds are easily split up and, aided by atomic migrations, others of a more solid structure

(1) From the dawn of exact knowledge to the present day, observation, experiment, and speculation have gone hand in hand. The invention of verifiable hypotheses is not only permissible but is one of the conditions of progress (*Huxley*). L'hypothèse et le raisonnement ont aussi un rôle indispensable ; il faut le répéter, puisque certains esprits persistent à vouloir que l'on s'en tienne exclusivement aux faits (*Leo Errera*).

are formed: the products of fermentations. Amid this destructive activity proteids are built up within the cells and become organised into living protoplasm, what under favourable conditions is done with such *rapidity* that one cell yields by multiplication in 24 hours more than *trillion of new cells*.

The degradation of complex substances must precede the synthetic work in order that not only energy but also the requisite atomic groups may be obtained.

Although organic compounds of very *different* chemical constitution may serve for nourishment and development, there can be hardly any doubt but that in all cases the *same proteids* result in one and the same species, otherwise the structure and the functions of the protoplasm formed would show variations and thus new species would spring into existence with ease under the influence of the food. We find, however, the species of bacterium or of mould-fungus preserved, whether it is nourished on glycerol, glucose, or galactose, acetic or quinic acid; whether we offer leucine or betaine, asparagine or kreatine as the source of nitrogen.[1]

This experience teaches us that the formation of proteids must commence with atomic groups simple enough to be derived from substances widely different.

The nutritive properties depend, other things being equal, upon the chemical constitution. The more easily a compound is attacked by the cells the quicker is the development of new cells. Slight chemical changes may

(1) The characters of certain bacteria may, however, be modified by changing other *conditions of cultivation;* but whether such modifications would lead in course of time to new *species,* a point which would be of high

convert an indifferent compound into a nutritive one and this again into a poison, a fact, illustrated by the following series: CH_4; CH_3OH; CH_2O. Besides, whether a substance proves to be nutrient or poisonous will depend often upon the degree of concentration of its solution. Good nutrients, such as glucose, can be utilised in higher concentration than poorer ones, such as sodium acetate.[1] It is further a noticeable fact that maleic acid is very unfavourable compared with the isomeric fumaric acid, and that in certain cases the accumulation of methyl-groups in a compound diminishes the nutritive value, as we observe in comparing tetramethylglycol (pinacone) with glycol, or trimethylamine with methylamine.[2] Sources of carbon are: alcohols, aldehydes, ketones, acids, esters, bases; sources of nitrogen are: nitrates, ammonium-salts, amido-acids, amides, amines, ammonium-bases, nitriles; sources of sulphur are not only sulphates but also certain organic sulphur compounds.

In regard to the relative value as sources of carbon for *aërobic* bacteria, the following conclusions may be drawn from numerous experiments:

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importance in connection with the problem of evolution, remains to be investigated.

(1) Some very small differences exert an influence here upon certain species, as *Pasteur* and others have shown, with dextro, and lævo-rotatory modifications of various compounds. Further, the introduction of an amido-group into a molecule will in many cases increase nutritive value, while in some it has the opposite effect; thus, *e.g.*, acetone is a good nutrient while diacetoneamine is a very poor one (*O. Loew*, Centrbl. f. Bact., 12, 362).

(2) *O. Loew*, Centralbl. f. Bact., 12, 465; *Bokorny*, Chem. Ztg., 1896, Nr. 9. That *chemical* stability is increased by the accumulation of methyl-groups in a compound has been repeatedly observed. The most interesting case of the kind has recently been described by *Ietrenko*, Chem. Ztg., 1895, pag. 1880.

1) The nutritive value of acids is enhanced by the presence of alcoholic hydroxyl-groups, and that of alcohols increases with the number of hydroxyl-groups; thus, lactic acid is superior to propionic acid, and glycerol to propyl-alcohol.

2) The presence also of aldehyde or ketone-groups increases nutritive qualities, so that glucose is better than mannitol.[1]

3) Lower alcohols, such as methyl-alcohol, may be used in higher concentration than the higher ones, *e.g.*, amyl alcohol.

4) The lower members of the fatty series are more easily assimilated than the higher members; sodium acetate being far more so than sodium valerate.

5) Unsaturated cyclic compounds are generally not favourable; thus pyridine, antipyrine, and dimethyl-oxypyrimidine appear to be wholly unsuitable, and benzoic and salicylic acids are very poor sources of carbon, while phenyl-acetic acid, as containing a CH_2 group, is far better, and quinic acid, containing *a saturated* benzene-ring with 4 CHOH groups, is a very good source.[2]

The question which is the compound that serves as a *starting point for proteid formation*, can only be answered by taking into consideration the lowest compounds utilisable

(1) If we compare, *e.g.*, ethyl acetoacetate with ethyl acetate, both in 0·1 per cent. solutions, we observe a more rapid development of bacteria in the former than in the latter. *O. Loew*, Biol. Centralbl., 10, 585.

(2) In carrying out such experiments, mineral solutions should be prepared, containing 0·02% magnesium sulphate, 0·2% potassium nitrate or neutral ammonium phosphate and 0·1-0·2% neutral potassium phosphate. After addition of the organic nutrient to be tested the liquids are sterilised by heat or filtration through *Chamberland's* filter and inoculated. Organic bases are best neutralised with phosphoric acid; acids are best employed as sodium salts.

as nutrients, and, as methyl-alcohol, methylamine and methyl-sulphuric acid[1] serve the purpose, the *starting group* must contain only *one atom of carbon* in the molecule. And, further, since methyl-alcohol or methylamine, *as such*, do not serve for synthetic operations, transformation into a compound capable of condensation must take place, and this can only be *form-aldehyde, the same substance that forms by condensation various kinds of sugars.* Neither acetic, glycolic, nor amidoacetic acid can be utilised *as such*, but by oxidation may lead to the one compound that can be utilised, *viz*, to *form-aldehyde*; no other unsaturated atomic group could here result, suitable for synthesis. This oxidation in the cells may be expressed by the following equation in the case of acetic acid:

$$CH_3.COOH + O_2 = CH_2O + CO_2 + H_2O$$

We can thus understand why polyhydric alcohols and acids are so favourable, and why such substances are capable of nourishing certain bacteria endowed with *fermentative properties, even in the absence of air*, while compounds without the CHOH-groups can be used as food only in the *presence of air*, oxidation being then necessary to produce this CHOH-group or the isomeric form-aldehyde.[2] But can that conclusion be admitted if form-aldehyde is a poison? No doubt this seems a weighty objection, but if we consider how easily form-aldehyde is changed under condensation influences and how indifferent

(1) In this case, for obvious reasons, an alkaline reaction is necessary.

(2) Thus, *e.g.*, acetates or leucine can nourish facultative anaerobs, only if air has access, while tartaric acid can do so also in absence of air; the oxygen of nitrates cannot replace free oxygen, probably because oxidation then takes a course different from that required to form the starting group for proteid formation.

certain compounds of this aldehyde are, the objection no longer appears so serious; we have only to adopt the view that the form-aldehyde undergoes rapid transformations and that no molecule remains unchanged for a second.[1]

If we are correct in our inference we may express the general rule thus:—

Compounds that are easily oxidised or decomposed by bacteria and thereby yield form-aldehyde are good nutrients for them.

In this connection also it is an interesting fact that there exists a microbe species,[2] which can develop in nourishing solutions containing as sole organic substance the combination of form-aldehyde with sodium acid sulphite, $CH_2OH.SO_3Na$; or the compound which form-aldehyde yields with ammonia, the so-called hexamethylenamine. This microbe is also capable of utilising formates,[3] in which case a synthetic operation has to be assumed to occur, form-aldehyde being probably reached by an intermediate formation of glyoxylic acid:[4]—

$$2HCOOH = HCO.COOH + H_2O$$
$$HCO.COOH = H_2CO + CO_2$$

A still farther reaching synthesis has to be accomplished when ammonium carbonate serves as nutrient for the nitrifying microbe, a highly interesting case, first observed by *F. Hüppe* and *Heräus* and studied also by *Munro*,

(1) Cf. *O. Loew*, Ber. d. Deutsch. Chem. Ges., 22, 484. Synthetic processes require substances of a certain lability; very active substances, however, are more or less poisonous.

(2) I described it as *Bacillus methylicus* in Centralbl. f. Bact., 12, 462.

(3) *O. Loew*, ibid., p. 463.

(4) Cf. *Koenigs*, Ber. D. Chem. Ges., 25, 801. *Bokorny* (*l. c.*) has observed that glyoxylic acid can be utilised by bacteria.

Warington Frankland, and *Winogradski.* Here, very probably, a part of the hydrogen of the ammonia serves for the production of form-aldehyde from carbonic acid.[1]

Some additional remarks may be made on the nutrition of mould-fungi (*Penicillium, Aspergillus, Mucor*). Substances supporting the life of aërobic bacteria, generally also serve as food for mould-fungi, though there exist exceptions: methylamine, methyl alcohol and sodium valerate are better utilised by bacteria, glyoxal better by mould-fungi. A neutral reaction is best suited for most kinds of fungi; in alkaline liquids, however, bacteria thrive better than mould-fungi, while in an acid one the contrary is observed (with some exceptions).

To those compounds which cannot be utilised by mould-fungi belong maleïc,[2] citraconic, mesaconic, dibenzylmalonic, and diethylsuccinic acids. Benzylsuccinic, di-substituted glutaric and oxyisobutyric acids are very poor sources, while malonic, succinic, monomethylsuccinic, and monoethylsuccinic acids are good ones.[3]

But it is not only in regard to the *sources of carbon* that great variety exists; it does so also in regard to the *sources of nitrogen.* Of the great number of the latter compounds I will mention, as examples: glycocoll, asparagine, kreatine, allantoine, methylamine, acetamide, methyl-cyanide, betaïne, strychnine. Nitrites are, in a certain concentration, less favourable than nitrates, and are in *acid* solutions poisonous. Hydroxylamine and diamide being

(1) Cf. *F. Hüppe,* Naturw. Einführung in die Bacteriologie, Wiesbaden, 1896, pp. 63-66.

(2) *E. Buchner,* B. d. Deutsch. Chem. Ges., 1892, p. 1163.

(3) *B. Meyer,* Ibid., 1891, p. 1071.

strong poisons, cannot be utilised at all, and hydrogen azide only in high dilutions.[1] For the reasons that hold good in the case of carbon, nitrogen compounds must be transformed first into one and the same atomic group, before synthetic work can start; this group is evidently *ammonia* which, in form of its salts, is not only very suitable for mould-fungi and bacteria, but is also the simplest nitrogen compound that can be directly utilised.[2]

The liberation of the nitrogen of organic compounds as ammonia may be accomplished by hydrolysis, as with amides; by oxidation, as probably in the case of amines and amido-acids; or by reduction, a means applied by *anaërobic* microbes. Compounds easily decomposed, as kreatine, will also, of course, be of more value than such as offer considerable resistance, as strychnine.[3]

Finally, in regard to the sulphur of the proteids,[4] the

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(1) *O. Loew*, Biol. Centralbl., 10, 588 and Ber. Deutsch. Chem. Ges., vol. 24, p. 2947. Certain fungi, as *Saccharomyces Mycoderma* prefer ammonia as source of nitrogen to amido-acids and peptone (*Beyerinck*). The common beer-yeast however, can at *low* temperature, make better use of the latter than of the former; further it cannot utilise nitrates (*A. Mayer*). *Laurent* has shown that this is due to the conversion of these into the poisonous nitrites.

(2) Nitrates have to be reduced first to ammonia. The supposition that *hydroxylamine* is the lowest nitrogen compound from which proteid-synthesis takes its start, is not admissible; for, leaving other objections aside, anaërobs would certainly find it impossible, to produce this compound from ammonia in absence of air.

(3) A very remarkable case is that of the assimilation of *free nitrogen* by certain bacteria of the soil, recognised years ago by *Berthelot* and recently confirmed by *Winogradzki*. The free nitrogen is here probably first converted into ammonium nitrite, and the nitrous acid then so rapidly reduced to ammonia, that it cannot accumulate or be set free.

(4) The proteids of mould-fungi and yeasts contain sulphur, but those of several kinds of bacteria were found free from sulphur by *Nencki*.

behaviour leaves no doubt that this is present in a *reduced* state, and hence the next conclusion is that all sulphur-compounds have to be reduced to hydrogen sulphide in the process of proteid formation. Thus, then, the experimental evidence leads us to the inference that the atomic groups for the production of proteids are :— *form-aldehyde, ammonia* and *hydrogen sulphide.*[1]

B. Nutrition of Phænogams.

As chlorophyll-bearing plants produce by assimilation carbohydrates, it is natural that such well-suited compounds should form, here also, the main source of carbon for the proteids. Nitrates or ammonium salts furnish the nitrogen ; sulphates the sulphur. If all conditions are otherwise favourable, then the synthetic work proceeds so rapidly that the intermediate steps cannot be directly traced. From numerous observations, however, the conclusion appears justified that it is *asparagine* to which an important rôle must be attributed in this connection.[2]

On the one hand, this substance appears as a final product of metabolism, on the other, as a step in the formation of proteids. We find it not only in shoots and buds and in plants kept in darkness but also in roots of

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(1) These three substances are either formed only in the quantities required for proteid-formation ; or, if an excess of one or the other should have resulted, are transformed into innocuous compounds. But certain species of bacteria produce ammonia, from amido-compounds or nitrates, and hydrogen sulphide, from sulphates, in excess of the needs for the production of proteids. Further, it may be assumed as highly probable that even the smallest excess of form-aldehyde is at once transformed into carbohydrates (mucilage, glycogen, etc.).

(2) *Th. Hartig* first recognised the importance of asparagine for proteid production. *Borodin, Pfeffer,* and *Kellner* declared asparagine to be

fully developed plants under normal circumstances. In shoots, the more of it is produced, the smaller is the amount of carbohydrates in the seeds[1] and, when these young plants have formed enough chlorophyll for the production of a larger amount of sugar by assimilation it disappears gradually again.

Moreover, before the assimilation process has set in with sufficient intensity, a gradual *increase* of asparagine is observed in proportion as the other amido-compounds, formed by the action of a trypsin-like enzyme upon the reserve proteids, *decrease.*[2] That the proteids, as such, are oxidised *dircetly* to asparagine, as *Boussingault* first supposed, has not been proved.

In those cases, however, in which roots and stems *rich in sugar* contain much asparagine, this cannot be derived from proteids by metabolism, and its origin has to be looked for from another source. It was surmised by various authors, *Kellner, Schulze,* and others, that nitrates or ammonium salts may give rise to its formation, but it has only recently been proved that it is essentially

the form in which proteids are translocated in plants. The most extensive investigations in this direction, however, we owe to *E. Schulze* and his school; cf. Landw. Jahrb., vol. 12; 17; 21. Landw. Vers. St., 1880–96.

(1) The degree of proteid decomposition in shoots depends largely also upon the *time,* at which the reserve-carbohydrates become *soluble;* only *dissolved* carbohydrates afford protection to proteids; in this way, certain anomalies observed in germinating peas as compared with beans may be explained.

(2) This way of generation of asparagine from proteids offers a certain analogy to that of urea in animals; in both cases amido-acids are the intermediate products which lead upon further oxidation to ammonia. In *animals* this again leads to carbamic acid (*Drechsel* and *Abel*), and thence to carbamide, whilst in *phænogams* it leads to asparagine. The investigations of *Nencki* and his collaborators have thrown a flood of light upon the production of urea in animals.

the latter which do so.[1] Nitrates can be stored up as such, if more has been absorbed than is immediately needed for proteid production, but ammonium salts cannot, being noxious in a certain concentration, and, therefore, have to be transformed into an innocuous compound; this proved to be *asparagine*.

Let us turn now to the question of the *conversion of asparagine into proteids*. The relative amount of sugar, the temperature, the intensity of respiration and of growth, the supply of the required mineral salts, all influence this process. We cannot, therefore, be surprised to see in cases where one of these conditions is wanting or imperfect, that asparagine may be present along with a considerable amount of sugar, without proteid production being accomplished. The lower temperature of the soil at a *certain depth*,[2] as well as the weaker degree of respiration (due, partly, to this lower temperature, partly, to the less favourable anatomical structure), brings out the result that in *roots* this process is much slower than in *leaves*.[3] This circumstance gave rise to misconceptions and even to the assertion that the transformation of asparagine into proteids would be possible only by the "nascent carbohydrates" in the chlorophyll bodies during the assimilation process, a hypothesis which would encounter more than one physiological objection that the thoughtful reader

(1) This question was examined into in the College of Agriculture of the Imperial Univerity, Tōkyō, by *Mr. Kinoshita* and *Mr. Suzuki*, who undertook its solution at my instigation. Under certain conditions, nitrates are quickly reduced to ammonia and will yield then, of course, also large quantities of asparagine.

(2) Only the superficial layers acquire a higher temperature by direct insolation.

(3) Stems and roots may serve also as reservoirs and for this reason also contain generally more asparagine than leaves.

will easily divine. Besides, recent experiments have proved beyond a doubt, that shoots can, even in complete darkness,[1] form proteids from asparagine when a suitable non-nitrogenous nutrient, as glycerol, is present in the nourishing solution. Thus, shoots of soya beans, deprived of their cotyledons, reduced their amount of asparagine from 21 to 13 per cent. within 27 days, when kept in solutions containing 1 percent of glycerol and exhibited a much better development than the shoots in water, which showed, moreover, an increase of asparagine from 21 to 28 per cent.[2]

How, then, are we to explain the transformation of asparagine into albumin? If we reflect that asparagine is of a low order of chemical compounds, while proteids are the most complex of all; if we take into consideration that there are encountered neither nitrogenous by-products nor any other intermediate substances; and, finally, if we observe the great rapidity of proteid formation[3] under favourable circumstances, we cannot but draw the inference that this remarkable transformation must consist in a so-called *condensation-process*. That is to say, asparagine being incapable of serving as such, has to be transformed into a suitable derivative exhibiting the same proportion between carbon and nitrogen atoms as albumin, *viz.*, 4 : 1. This looked-for product can hardly be any other than the

(1) It is an old observation also that fungi can prepare in complete *darkness* their proteids from various sources.

(2) *Kinoshita*, Bulletin of the Agricultural College of the Imperial University, Japan, vol. II., no. 4.

(3) A bamboo-shoot will grow in sub-tropical regions one centimeter per hour, in tropical regions much more; which signifies the production of millions of molecules of albumin in a single cell, every minute.

aldehyde of aspartic acid, a still unknown product, whose exceedingly changeable nature can be foretold.[1]

Referring to what I have pointed out above, that formaldehyde and ammonia, besides hydrogen sulphide, are the groups serving to build up proteids, aspartic aldehyde would consequently represent the following step, a transition which thus far has not been accomplished. Supposing, however, the living cells, to bring this on, it could be represented by the following equation :—

I. $$4CH_2O + NH_3 = \underbrace{\begin{matrix} COH \\ | \\ CH_2 \end{matrix} - \begin{matrix} COH \\ | \\ CH.NH_2 \end{matrix}}_{\text{Aspartic aldehyde}^2} + 2H_2O.$$

The subsequent condensations may be represented by the following equations :—

II. $$\underbrace{3C_4H_7NO_2}_{\text{Aspartic aldehyde}} = \underbrace{C_{12}H_{17}N_3O_4}_{\text{Intermediate product}} + 2H_2O;$$

III. $$6C_{12}H_{17}N_3O_4 + 12H + \underbrace{H_2S = C_{72}H_{112}N_{18}SO_{22}}_{\textit{Lieberkühn's}\text{ albumin-formula}^3} + 2H_2O.$$

(1) I first drew this inference in the year 1880 (*Pflüg.* Arch., 22, 503), in opposition to the generally adopted opinion that proteids are complex ureas or guanidines, containing the radicals of those amido-compounds that result from the decompoition of proteids by acids. Those who uphold this view have to assume that all those amido compounds have to be prepared first in the proper quantity, whereupon they are forced at the proper moment and in the proper order into the urea or guanidine-type (cf. Chapt. III), certainly a most complicated sort of work.

(2) *Schützenberger* has met with a body of this empirical composition among the decomposition products of proteids by caustic baryta; *Siegfried* with a similar one, to which he assigned the formula, $(C_4H_8NO_2)_n$.

(3) It is certainly no accident that the number of carbon atoms for the lowest possible expression of albumin stands in a simple ratio to those in the molecule of glycerol, sugar, stearic acid, and oleic acid. We have C_3 in glycerol, C_6 in glucose, C_{18} in stearic and oleic acids, and C_{72} in the simplest expression for albumin. This circumstance points also to the principle of condensation after simple ratios.

For the process represented by equation II., the assumption has to be made that the amido-groups are protected, and that the condensation proceeds between the aldehyde and methylene groups, while for that in equation III., condensation by reduction after the type of pinacone-formation is assumed. In this way, a highly *unstable* substance would result containing 12 aldehyde and 18 amido-groups, and possessed, therefore, of much kinetic energy in the form of motions of the labile atoms; while, by *atomic migration*, it would with the loss of the aldehyde character lead to a relatively *stable* product, the ordinary albumin.

Let us now, guided by our hypothesis, review the observations in regard to the occurrence of asparagine in plants. The accumulation of it in developing shoots and buds is intimately connected with the gradual consumption of the amido-products (leucine, tyrosine, phenylamido-propionic acid, glutamic acid, aspartic acid, lysine, arginine), which result from the action of an enzyme upon the reserve proteids. These amido-products, either on their way to the young growing parts or, after their transportation thereto, are gradually disappearing, while proteids are formed for the protoplasm of the growing and multiplying cells. All these different products, however, have to be either transformed into form-aldehyde and ammonia or more directly into aspartic aldehyde,[1] before proteid formation can take place, as this process, can hardly go on here differently from what it does in the fungi; cf. p. 33.

Thus the decomposition of leucine by oxidation may be expressed by the following equation :—

(1) Aspartic acid and glutamic acid probably are transformed directly into aspartic aldehyde.

$$C_6H_{13}NO_2 + 7O = 2CO_2 + H_2O + 4CH_2O + NH_3.$$

Thus, not only are the starting groups obtained, but respiration is supported in the combustion of a part of the carbon and hydrogen of leucine. If, now, less carbohydrate is present than is required for the transformation of all the ammonia, thus formed, into proteids, then asparagine[1] will result :—

$$4CH_2O + 2NH_3 + O_2 = \underbrace{C_4H_8N_2O_3}_{\text{Asparagine}} + 3H_2O$$

If, however, glucose or other suitable material is brought in sufficient quantities from the reserve stores, or from the green leaves, then an adequate amount of this asparagine is transformed into proteids by way of aspartic aldehyde, which we may express by the general equation :

$$\underbrace{C_4H_8N_2O_3}_{\text{Asparagine}} + C_6H_{12}O_6 + O_2 = \underbrace{2C_4H_7NO_2}_{\text{Aspartic aldehyde}} + 2CO_2 + 3H_2O$$

Further, in those cases, in which, as in the seeds of *Gramineae*, there is a considerable excess of carbohydrate present as reserve material, an intermediate production of asparagine will take place only to a very small extent, as the proteids can be formed more directly in the shoots.

Finally, in the instances, in which asparagine is not the result of metabolism but of a product due to an excess of ammonia taken up by the roots, the necessary carbon in the form of form-aldehyde is in all probability derived from glucose, and thus the formation of asparagine by synthesis appears exactly the same process as when formed by the destruction of proteids, although the original material is vastly different.

(1) Thus, two noxious compounds are transformed into an indifferent one.

To the inferences to which I have been led by contemplation of the facts, further belongs the conclusion that the definition of *Pfeffer*, *Borodin*, and others, of *asparagine as the form in which proteids are transported*, does not hit the point, since the plant cells produce their proteids also from other amido-compounds. But as the various amido-products formed by the action of enzymes upon proteids, leucine, tyrosine, *etc.* are, on the way to their destination, partly oxidised, whereby their nitrogen accumulates in the form of asparagine, it gives the impression that the transportation of proteids takes place in this form. The facts, however, are better covered by the definition of asparagine as the form into which *ammonia is transformed* when it is present in excess of the immediate needs of proteid production ; it appears also as the form into which aspartic aldehyde is converted when an excess of ammonia is present, and thus a step in the proteid production is preserved in the form of a derivative.[1] The relations between arparagine and albumin will appear now more simple than they did from the former views, which evidently disregard the principle of economy of work.[2] *The important part too, which glucose plays* is easily intelligible. It is, in the first place, an excellent source of carbon and hydrogen, secondly, a ready means of reduction, and, thirdly, the best supporter of respiration by which an increased amount of energy for chemical work is procured. Finally, it protects proteids, to a *certain extent*, against disruption. It is therefore clear that *the leaves* are so well suited for the process of proteid produc-

(1) Asparagine, however, is not only an excellent nutrient for phænogams but also for fungi.

(2) Critical comments upon other views are contained in *E. Schulze's* publications, Landw. Jahrb., vol. 9; 12; 17; 21.

tion, although the assimilation process, *as such*, *i.e.* a *direct* influence of the sun's rays, is not requisite.

From a series of well established facts and guided by simple chemical laws, I have framed a hypothesis as to the formation of albumin, and as to the existence of a labile and a stable modification of it. The labile form which would lead to living matter, was designated by myself as *active* albumin, in contradistinction to the stable, ordinary, *passive* albumin stored up in seeds and eggs. The name 'living albumin' used by some authors cannot signify any thing but living protoplasm and had better be discarded altogether to avoid misconceptions. Still more objectionable for the molecules in the living protoplasm is the expression 'living molecules' and 'units of life.' The name 'active albumin' on the other hand, does not necessarily imply organisation, and is a mere chemical conception.[1] Indeed there exists a highly labile proteid in plants which is not yet organised into living matter; the nature of this is the subject of the following chapter.

(1) Since there must exist energetic atomic oscillations in labile substances this designation will be found justified (cf. Chap. VI).

CHAPTER V.

ACTIVE ALBUMIN AS RESERVE-MATERIAL IN PLANTS.

The hypothesis developed in the foregoing chapter induced *Dr. Th. Bokorny* and myself to make a series of experiments on vegetable life[1] whose final results may be summed up as follows :—

1. There exists in plants a peculiar albumin that undergoes a chemical change by the same influences as those by which protoplasm is killed.
2. This albumin plays the part of a reserve material.
3. It can occur in higher as well as in lower forms of chlorophyllous plants, is to be found in various parts and some organs at all times, and again may be restricted to certain states of development.
4. It is by its labile nature strikingly distinguished from the ordinary proteids.

This peculiar, easily changeable proteid is met with in certain groups of plants very frequently, as in *Juliflorœ, Cistiflorœ, Æsculineœ, Saxifragineœ, Myrtiflorœ, Rosiflorœ, Bicornes.* It has not yet been found in *Poa, Hordeum, Avena, Pisum, Vicia, Solanum.* In fungi[2] and most algæ it has also not been detected, but certain algæ, especially *Spirogyra,* are capable of storing up large quantities of it. Its wide-spread occurrence in the vegetal

(1) Cf. Botan. Centralblatt, 1889 and 1893; Flora, 1892 and 1895 *Pringsheim's* Jahrb., Vol. 19 and 20; *Pflüg.* Arch., vol. 45 and 50.

(2) Slight indications of it in certain cases require further examination.

kingdom may be estimated by the fact that, of 230 species examined by *Th. Bokorny*, *G. Daikuhara*, and myself 120 were found to contain it in one part or other of their structure.

Of special objects rich in this proteid may be enumerated: young leaves of *Prunus*, *Rosa*, *Quercus*, *Alnus*, *Mimosa*, *Pæonia*, *Saxifraga*, *Sedum*, and *Cephalotus*; the bark of *Prunus*, *Quercus*, and *Fagus*; petals of *Gentiana Primula*, *Sorbus*, *Cyclamen*, *Hotteia*, and *Cornus*; stamens of *Eugenia*, *Drosera*, and *Melaleuca*; pistils of *Crocus*, *Salix*, *Euphorbia*, and *Rhododendron*; nectaria of *Passiflora*; roots (epidermis) of *Saxifraga*, *Œnothera*, *Thesium decurrens*, and *Xanthoxylon piperitum*; fruits (epidermis) of *Punica* and *Camellia*.

This proteid can be present in the different tissues of the leaves, but cells of epidermis and of the fibro-vascular tissue are in certain cases preferred by it. Young leaves contain generally more of it than older ones, but even when leaves have turned yellow in autumn it may yet be there, provided the cells are still alive. In roots and fruits it seems to occur less frequently than in leaves and flowers. Leaves in the shade contain less than those in the light, while leaves with partial albinism may contain in the white parts nearly as much as in the green. In plants exposed to starvation by being kept in darkness, it is gradually consumed, with production of amido compounds.[1] Of parts that contain it only at one stage of development, may be mentioned: the unripe fruits of *Symphoricarpus racemosus*, the cotyledons of *Helianthus*, the epidermis of the seeds of *Triticum*, the larger cells in the leaves of *Vallisneria*. Especially noticeable is the large amount

(1) Cf. *O. Loew*, *Flora*, 1895. p. 85; *Daikuhara*, ibid.

present in the insectivorous plants, *Utricularia* alone being devoid of it. *Drosera* shows it not only in the leaves and their tentacles, but also in stem and flower.

Active albumin is stored up generally in the vacuole, but in some cases also in the cytoplasm, and is separable from its apparent solution by organic bases. If the bases applied are weak enough not to injure the life of the cells in too short a time, the small liquid particles of the separated albumin will preserve for some time their chemical properties and coalesce gradually to larger, bright droplets, whose changes by various reagents can easily be followed under the microscope. Caffeine and antipyrine in 0·5 per cent. solution answer the purpose very well.[1]

Generally, it will suffice to place small chips of the vegetable tissue in a few drops of caffeine solution and then tear them up into finer fragments by the help of dissection needles. Microscopical examination will then reveal either at once or, in case of thicker cell-walls being present, after some time, numerous globules uniting gradually to larger ones, which may occupy one fourth and more of the entire volume of a cell.

These globular formations were designated by *Th. Bokorny* and myself as *proteosomes*.[2]

If the objects are taken from these solutions and replaced in pure water, the droplets will gradually disappear again

(1) Caffeine acts in much higher dilutions than antipyrine, and is in many cases preferable, because it does not interfere with certain chemical reagents to be applied for identification, such as iodine solution, which would yield with antipyrine a dark brown coloration.

(2) Some remarks may here be made as to the aggregation, observed first by *Ch. Darwin* in the leaves of *Drosera*, in which the living protoplasm itself, as well as the stored up active albumin, is involved. *De Vries*, *Pringsh.* Jahrb., 16, has recognised that here, as in other cases, the division

in proportion as the bases mentioned leave the cells by osmosis. The cells then continue their life as before the treatment. This dissolving process is accelerated by higher temperatures; at 30°C. it requires but a few minutes. A return of the objects to a solution of one of these bases makes the droplets reappear. If, however, the cells *die* by the prolonged influence of caffeïne or antipyrine, or if they are *killed* by iodine solution, or acids, or by form-aldehyde, hydroxylamine, diamide, prussic acid, free cyanogen, or salts of copper, or by vapours of ether, then *these droplets change their properties*, becoming turbid from numerous and minute vacuoles, formed by a sudden loss of absorbed water, and *losing their solubility* in water[1]. In some cases the small vacuoles unite into one large one, a hollow sphere thus resulting, in other cases the spherical form is lost entirely, leaving an irregular shaped mass. In others, again, the vacuoles disappear by further contrac-

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of the contracting tonoplast (the inner wall of the cytoplasm) can produce globular formations, what he called *anomalous plasmolysis*. *Bokorny* declared (*Pringsh.* Jahrb., 20. 465, and Biol. Centr., 13, 231) that the production of proteosomes has the principle in common with ordinary and anomalous plasmolysis that some of the water of imbibition is expelled, and it is an interesting fact that he could produce all three phenomena with even very dilute caffeine solution, and sometimes two of them in one and the same object. Plasmolysis, by caffeine can, *e.g.*, be observed in the petals of *Ipomœa hedracea*, the leaves of *Camellia theifera*, the leaf-veins of *Pyrus Toringo*, and sometimes in *Spirogyra*, after it has been cultivated in solutions containing at least 5 p. mille mineral salts. By treatment with dilute ammonia (0·2%) or iodine solution, plasmolysed formations can easily be distinguished from proteosomes.—Another closely related phenomenon is the enlargement of vacuoles under the influence of caffeine in amœbæ and infusoria, whereby the protoplasm increases in density and refractive power, as observed by *Bokorny*, *Pflüg.* Arch., 59, 557.

(1) It is but rarely the case that the proteosomes are altered *before* the death of the cells is caused by the prolonged action of caffeine.

tion, and the solidified but now insoluble globules retain a certain brightness. In one case, viz., that of the proteosomes produced in the petals of *Hotteia japonica*, still another phenomenon has been observed, consisting in the formation of numerous radiating fissures by treatment with a dilute solution of iodine in potassium iodide. It offers considerable interest to watch these changes under the microscope, as *e. g.* that caused by alcohol of 10–20 per cent., or by hydrochloric acid of 10 per cent., form-aldehyde of 10 per cent., prussic acid of 1–2 per cent. Acetic acid of 0·1 per cent. will, although more slowly, also produce coagulation.[1]

Still more striking is the effect of ether vapour. If *Spirogyra*, which is an excellent object, containing freshly produced droplets, is exposed in a flask at the ordinary temperature to the vapours of ether, the cells are found killed in a short time, and soon afterwards all the globules change their aspect, losing their brightness and their solubility.

The coagulation by heat is easily observed if the objects are dipped in boiling water containing 5 per cent. of sodium chloride, all droplets then exhibiting a turbid appearance; neither boiling water nor absolute alcohol will change them any further. In a saturated caffeine solution

(1) If, instead of diluted alcohol, *absolute* alcohol is applied, the caffeïne is so rapidly extracted that the smaller globules *dissolve* before they are coagulated, while the larger ones shrink to irregular-shaped, thin films. Here, the exosmose of caffeïne proceeds more quickly than the endosmose of a sufficient quantity of alcohol. *All these experiments are best made with the larger globules;* the changes cannot be well observed if the globules are too minute; for then they may be dissolved before the proper action can take place, especially in the case of dilute acids. Therefore, the caffeine solution should be permitted to act until the smaller globules have united to droplets.

containing 5 per cent. potassium sulphate, a gradual coagulation is observed at 50°.

Of much interest to chemists is the behaviour to ammonia.[1] While freshly produced proteosomes are *dissolved* by *concentrated* ammonia, they will turn *insoluble*, if exposed for 12–15 hours to the action of ammonia in the high dilution of 0·1–0·5 per mille. This change, however, is different from that produced by heat or acids, as the chemical behaviour clearly indicates.[2] A dilute hydrochloric acid (0·5 per cent.) will at 40°C. gradually attack the coagulated proteosomes, but not those solidified by ammonia. Even a 10 per cent. hydrochloric acid dissolves the latter at 80° with much difficulty.

Active albumin also changes quickly in the *dissolved* state with the death of the cells; in dead cells caffeine never produces any globules.[3] If we treat *Spirogyra Weberi* for *one* minute with a dilute solution of iodine in potassium iodide the globules may be still produced immediately afterwards, but not after ten minutes action.

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(1) This behaviour to ammonia may serve in certain cases to distinguish small and indistinct proteosomes from tannates of caffeïne or antipyrine, which will be readily dissolved by dilute ammonia; when proteosomes are very rich in tannin the behaviour to ammonia may be altered.

(2) All these reactions are so characteristic that confounding proteosomes with fat globules is not possible. The simplest ways to distinguish them are, firstly, the application of dilute solution of iodine in potassium iodide whereby a vacuolisation of the proteosomes readily sets in; secondly, treatment with alcohol of 20 per cent. for one hour, with subsequent application of a mixture of ether and alcohol, which does not affect the coagulated proteosomes.

(3) The opinion that the change of the active albumin in the vacuole is brought on by certain compounds that pass from the cytoplasm into the vacuole at the moment of death, is erroneous, since the same labile proteid is contained also in the cytoplasm in the case of *Spirogyra*.

That the substance has not left the dead cell by osmosis can be easily proved by caffeine.

It is then an undeniable fact that the albumin here described is strikingly distinguished from ordinary albumin, although otherwise it gives (in the coagulated state) all the reactions of it.[1]

Treated with phosphotungstic acid the proteosomes remain insoluble even after weeks, while hydrochloric acid of 10 per cent. changes them gradually and dissolves them in some days at the ordinary temperature.—A solution of caustic soda or potash of moderate strength soon dissolves the proteosomes, but if they have been treated with a neutral solution of form-aldehyde they have lost their easy solubility in caustic lyes,—in this recalling that behaviour of ordinary proteids, I observed some time ago.[2]—*Millon's* reaction is obtained by leaving the objects for 8–10 hours in a solution of mercuric nitrate containing some potassium nitrite, and then heating for a short time to the boiling point.—The 'biuretreaction' is obtained on treating the proteosomes, first, for twelve hours with diluted ammonia (0·1%), then, for 12 hours with a dilute solution of copper acetate, and, finally, with dilute caustic potash.

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(1) Instructive slides for demonstration may be produced by treating objects (filaments of *Spirogyra*) for several hours with 0·5% caffeine solution, then, for 12 hours with a solution of 0·01% ammonia, and lastly after extraction of the chlorophyll by ether-alcohol, with a highly dilute solution of methyl green in acetic acid. Remarkable is the great brittleness of these solidified proteosomes, for slight pressure will break them into numerous fragments. This recalls the striking brittleness of blood crystals (*Preyer*, 1871) and of the crystallised phycocyan, a proteid recently studied by *Molisch* (Bot. Ztg., 1895, p. 133).

(2) Cf. *O. Loew*, Jahresbericht f. Thierchemie, 1892, p. 29. and 1888, p. 273.

The proteosomes give also an intense yellow colour with hot nitric acid, are coloured yellow by iodine, and have the property of being easily stained by aniline dyes, in even very high dilution. In short, their proteic nature is proved.

That this labile proteid corresponds in essential points to my theory cannot be doubted, since it can be demonstrated (1) that it serves for building up new protoplasm, and (2) that it has an aldehyde character. That it serves for building up protoplasm can easily be verified by cultivating *Spirogyra*, rich in this labile reserve proteid, in solutions devoid of any assimilable nitrogen compound, but otherwise well suited to promote multiplication of cells. Any formation of proteid is then impossible, and the growing filaments have to draw upon the store of labile proteid in the cells; that they do do so can be shown by the caffeine reaction.[1] The increase of living matter is here most probably accomplished by direct 'organisation' of the labile reserve proteid. A previous coagulation, decomposition into amido acids, and transformation of these into living matter would be an unnecessary, time-absorbing operation, contradicting the principle of economy of work; the transportation through various cell strata required by

(1) Growth of the filaments, connected with total consumption of the labile reserve proteid, can, *e.g.*, be observed on *Spirogyra Weberi*, cultivated for several weeks in the following solution:—

0·5 per mille calcium sulphate.
0·2 ,, ,, calcium carbonate (as bicarbonate).
0·2 ,, ,, magnesium sulphate.
0·05 ,, ,, monopotassium phosphate.
Trace of ferrous sulphate.

As soon as parasites (*Pseudospora*, *Chytridia*, *etc.*) make their appearance, the filaments have to be washed and the solution renewed.

a growing shoot, is not at all necessary here, where each growing cell is provided with a certain amount of labile reserve albumin.

If, however, we change the conditions, reducing the amount of magnesia, and offering a large proportion of nitrates,[1] we can observe again, after a few weeks, an intense formation of proteosomes by the caffeine treatment.

Changes of temperature have also great influence. Hot weather favours multiplication of cells, and then the active albumin is consumed as rapidly as it is produced. If, now, cold weather follows, growth will be more retarded than the production of proteids, and that leads again to an accumulation of the active albumin in the unorganised form.[2]

As to the question whether the labile albumin has also the aldehydic character, required by my hypothesis, the following observations may serve to answer it. We know that, of compounds not of an acid character,[3] only aldehydes

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(1) I made use of the following composition :—

0·5 per mille potassium nitrate.
0·3 ,, ,, calcium nitrate.
0·05 ,, ,, magnesium sulphate.
0·05 ,, ,, monopotassium phosphate.

Slight trace of ferrous sulphate.

Spirogyra majuscula forms in this solution, after several weeks, such large quantities of active albumin, that it commences, in some cells, to separate in globules, *even without the caffeine treatment.*

(2) The state of *copulation* does not depend upon the amount of stored up active albumin ; I have observed, in some instances, much of the latter, in others, none at all.

(3) The contents of living cells of *Spirogyra* have not an acid reaction ; otherwise, diluted solutions of potassium iodide or nitrite would prove poisonous, as is the case with all plants that have an acid cell sap. However, by the change from life to death, caused by heat or by mechanical action, the weak alkaline reaction of the protoplasm becomes still weaker, or even slightly acid.

and ketones can readily combine with ammonia; and that, in certain cases, ammonia can react in such a way with these bodies as that it cannot again be liberated.[1] I have pointed out above that, by the action of ammonia, the proteosomes undergo a change quite different from that produced by acids or heat and that we must infer that it enters into most intimate combination with them, since, otherwise, treatment with hydrochloric acid should suffice to extract it again and yield thereby the same coagulated product that is obtained by the direct action of hydrochloric acid upon the caffeine proteosomes. The ammonia must, therefore, have converted the labile proteid (at least partially) into a product different in character from those which result when it acts upon mono aldehydes.

While caffeine or antipyrine forms evidently a very loose kind of combination with the labile reserve proteid, or leads to a loose kind of polymerisation, (with partial loss of water of imbibition), that allows the separated labile proteid to preserve the liquid form; stronger bases, though at first also producing granulations, change these so quickly that no coalescence into droplets can take place, and besides, soon kill the cells. Ammonia acts thus if highly dilute, say 0·2–0·5 per mille, while in a concentrated state it fails to produce a trace of granulation because the dissolved labile proteid is too rapidly altered. Amines, ammonium bases, piperidine, and quinine act nearly like ammonia, while quinoline, pyridine, and morphine operate less energetically. Certain bases, as quinine, act also when in the form of various salts; others, as ammonia or guanidine,

(1) This is, *e.g.*, the case when ammonia, acting upon 1.4. diketones, produces pyrrols.

also in form of carbonates, while potassa or soda acts only when free. A decidedly basic character is essential; thus, *e.g.*, trimethylamine or amarine can produce proteosomes; betaine, kreatine, urea, or hydrobenzamide cannot. While this behaviour is quite in accordance with the supposed aldehyde nature of the labile proteid, such a constitution is rendered still more probable by the fact that the proteid *reduces the metal* from even highly dilute alkaline silver solutions.

This reaction requires objects *free of tannin*, which has itself reducing properties, although very small traces of it will not essentially interfere with the result. We observe in both cases an intense blackening of all the proteosomes by a prolonged action of a *highly dilute* alkaline silver solution, while after coagulation of the proteosomes this reaction is not obtained; but if a trace of tannin is present, a *yellow* coloration of the *coagulated* proteosomes will make its appearance under the action of the silver solution.

It is well known that various kinds of tannins are of wide-spread occurrence[1] in the vegetable kingdom; there still exists, however, a great difference of opinion as to their physiological relations.[2] In different species of algæ it is also present, but the amount of it, *e.g.*, in *Spirogyra* may vary considerably, as from several per cent. of the dry matter to none at all.

The fact that the tannins easily enter into combination with proteids, implies that, when proteosomes are produced by the action of bases, all the tannin present is drawn into the proteosomes.

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(1) Cf. *Henry Trimble*, The Tannins, 1894, 2 vol.

(2) Cf. *Reinitzer*, Ber. D. Bot. Ges., 1889; vol. 7, 187.

Further, it has to be noted that, although the tannin is produced in the living matter itself, it accumulates in the *vacuole*, into which it is secreted. Often there are mere traces of tannin in the *cytoplasm* and even these will easily disappear altogether by keeping the algæ for some time in soft well-water in darkness, while the tannin in the *vacuole* will not thus completely disappear, not even when death by starvation has set in. In such a case, it is easy to observe that the proteosomes produced in the *cytoplasm* reduce alkaline silver solution just as well as those produced in the *vacuole*.[1] For these observations *Spirogyra nitida*, a larger species is best adapted.

The decrease of tannin can be accomplished in full daylight under various conditions, which either depress its production or increase its consumption. The tannin of the cytoplasm then disappears always long before that of the vacuole.

Various circumstances, such as difference of species, degree of temperature, the amount of tannin originally present, *etc.*, will exert some influence upon the time required to free the cells from tannin. Smaller kinds of *Spirogyra* containing only one chlorophyll spiral are sooner obtained free of tannin than the larger kinds. Thus, a sample of *Spirogyra gracilis* containing so much tannin that the decoction made with relatively little water

(1) The two kinds of proteosomes can easily be distinguished by placing threads of *Spirogyra nitida* in a narrow tube in a vertical position while the caffeine acts; for, then most of the proteosomes formed in the vacuole will settle to the bottom of the cells, whilst those of the cytoplasm will remain *in situ*.

Sometimes there is in smaller kinds of *Spirogyra* so much of the labile proteid stored up in the *cytoplasm* that the production of proteosomes will lead to serious disorder and therefore an early death.

yielded a dark blue colour with ferrous sulphate, could by five days cultivation be so far deprived of tannin that not the slightest trace of colour could be obtained by that reagent.[1] The last remnant of tannin, which could not be extracted by boiling water, because it was retained by proteids, disappeared within the following three days.[2]

The solution used in this case was prepared by dissolving in the purest distilled water[3]

0·20 per mille monopotassium phosphate,
0·02 ,, ,, monopotassium carbonate,
0·10 ,, ,, calcium sulphate,
0·05 ,, ,, calcium nitrate,
slight traces of magnesium and ferrous sulphates.

The amount of the labile reserve proteid gradually decreased but a moderate portion was still there when the last trace of tannin had disappeared.[4]

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(1) Ferrous sulphate which is here a much better reagent than ferric chloride, will indicate tannin even in a dilution of 1 in 100 000.

(2) The last traces of tannin are best discovered by first producing proteosomes, then moistening the object with a solution of ferrous sulphate, and permitting the water to evaporate slowly. The dried objects are moistened again and, after exposure to the air for some time, examined with a high magnifying power. A trace of tannin will thus lead to a blue coloration of the proteosomes. In those cases in which the objects have been treated with acids to coagulate the proteosomes, a washing with highly diluted ammonia is recommended before the application of ferrous sulphate.

(3) As the common distilled water often acts noxiously because of the presence of traces of copper, it has either to be redistilled from glass vessels or left standing with some iron filings for 15-20 hours before it can serve for preparing the nutrient solutions.

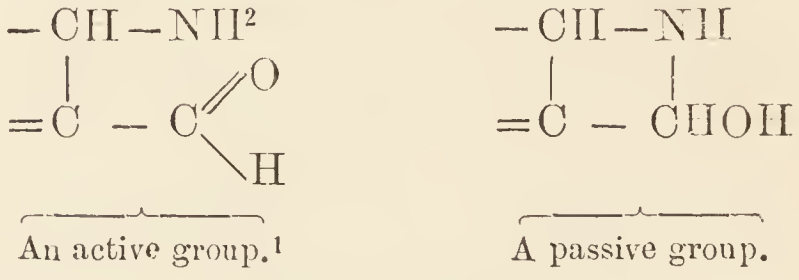
(4) There exist also other objects free of tannin, yielding proteosomes; among which may be mentioned the roots of *Thesium decurrens*, young hairs of many Crucifers, and the unripe berries of *Symphoricarpus racemosus*.

These cells yielded no trace of any reduction, either in cytoplasm or nucleus, or in the cell sap, upon being placed for 24 hours in the dark in a 1 per cent. neutral solution of silver nitrate; only the chlorophyll-bodies showed, along their rim especially, a yellowish colour. A 0·5 per cent solution of osmium tetroxide produced in all cells a weak blackening; and in many cells a very fine granulation could be distinguished exhibiting a deeper blackening than the other parts. This osmium reaction can only be due to traces of lecithin or perhaps more properly lecith-albumin. The chlorophyll bodies had preserved their green colour and could in this (perhaps exceptional) case not have contained any fatty matter.

Some filaments of this culture boiled with a few drops of water yielded a decoction which remained *colourless* upon addition of a 0.1 per cent. solution of silver nitrate supersaturated with ammonia; neither did the boiled filaments themselves show any reaction with this solution. Hence, neither a silver reducing body was present in the extract nor an insoluble reducing body *within* the *boiled* cells. Nevertheless, the proteosomes, produced with caffeine in the *living* cells, exhibited an *intense blackening* if kept in a highly dilute alkaline solution of silver nitrate[1]

(1) This solution was prepared just before use. A 1 per cent. solution of silver nitrate and a mixture of 13 *cc.* potash ley of 1·33 sp. grav. with 10 *cc.* of aqueous ammonia of 0·96 sp. grav. were kept in stock. One *cc.* of each solution was taken, the two quantities were mixed and the mixture was diluted to one liter. About one fourth of a liter of this reagent served for the test with a few filaments. This high dilution was preferred in order to avoid the objection that the reducing atomic groups could be other than aldehydic ones. In the present case, only oxyketonic ones could also come into consideration, which are, moreover, closely related to the former. This reaction is of course quite different from the ordinary staining method with silver nitrate under the influence of light.

for 12–15 hours in complete darkness. When, however, these *proteosomes had been changed by coagulation, the reducing power had vanished.* My theory (cf. Chap. IV) explains this chemical change by an atomic migration, leading to the loss of the aldehyde group :



An active group.[1] A passive group.

That the entire 'active' group has exactly the constitution ascribed to it by my theory, is not indeed yet proved, but I refer to it, because this assumption it is which has given the inducement for the entire investigation.[2]

In order to demonstrate that by coagulation the proteosomes lose the property of reducing alkaline silver-

(1) Twelve of such groups are—according to my hypothesis of the formation of active albumin—contained in one molecule of the latter, if the simplest expression, *i.e.*, *Lieberkühn's* formula, for albumin be taken as a foundation.

(2) A few words may suffice here to characterise the objections raised against the explanation given by *Bokoruy* and myself of this silver reduction. It has been asserted that the reducing substance is free tannin, tannate of albumin, tannate of caffeine, or hydrogen peroxide. It has been asserted that the reaction indicates merely certain reducing processes going on in living cells, and that the reducing groups cannot be with any degree of certainty defined as aldehyde groups because also hydroxylamine, alloxan, and morphine have reducing properties. The thoughtful reader will, I surmise, not insist upon a reiteration of the refutations, which we were moved to make ; those who desire to control our arguments may be referred to *Pflüger's* Archiv, 30, 357 ; Ber. Deutsch. Chem. Ges., (1888) 21, 1848 ; Botan. Centralblatt, 1889. Nr. 18, 19, 39, 45; ibid., 1893. Nr. 6. *Flora*, 1895, p. 68. It will suffice to state, that not a single one of the objections was proved, but a liberal use was made of violent language and derision, in order to give apparent strength to feeble argumentation.

solution the following experiments with *Spirogyra*, in which proteosomes were produced by the action of caffeine, may serve :

1. The filaments are formed into a small heap and thus allowed to dry up very slowly. Hereby, all the small proteosomes unite gradually to a few droplets which finally, upon the death of the cells, turn into hollow spheres. These remain either *colourless* after 8-10 hours treatment with the silver solution in the dark, or become merely *slightly yellow*. If, however, the filaments are dried up *rapidly*, the numerous small globules do not unite, but shrink to bright particles, which still retain the reducing power, like those treated with ammonia (see below), thus indicating that the caffeine has entered now into *chemical* combination, whereby the aldehyde character has been but little altered ;

2. The filaments taken from the caffeine solution are placed in a cold saturated caffeine solution to which 20 per cent. alcohol has been added. After 10 hours, or even less, all the proteosomes are coagulated (vacuolised) and have completely lost every trace of reducing power. Ether vapours also may be applied for one hour with the same result ;

3. The filaments are pressed between two object-carriers, and left there, protected against drying up, for 10 hours.[1] Microscopical examination will then reveal dead cells with coagulated proteosomes,[2] and living cells with unchanged ones.

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(1) While the chlorophyll here preserves its colour, it turns yellow by the treatment with alcohol of 20 per cent.

(2) Here merely the contact with the *dead* protoplasm produces the coagulation of the proteosomes. In those cases in which small

Accordingly, we find, after the treatment with the silver reagent, that those coagulated remain colourless while those that have not been coagulated become intensely black.

The noticeable behaviour of the proteosomes to *ammonia*, pointed out above, has also considerable interest in regard to the silver reduction. By the fixation of ammonia the *reducing power* is not only retained for a longer time, but its destruction requires now also more energetic means. A slow drying up or treatment with a 1 per cent. acetic acid for twelve hours now proves insufficient to destroy it. Boiling water destroys it in about two minutes (a few seconds are not sufficient), acetic acid of 30 per cent. or hydrochloric acid of 10 per cent. in one to two minutes.[1]

Guided by my theory, this phenomenon finds a simple explanation, since aldehydes retain their reducing power after the fixation of ammonia.[2]

Moreover, this behaviour admits of a simple explanation of the apparently anomalous fact that while cells killed by heat, pressure, alcohol, or acids have lost the silver reducing property, those killed by diluted ammonia have not.[3] It explains, further, why cells brought, in a *living* condition, into an alkaline silver solution continue to reduce silver even after they have died in it. For, this solution contains *ammonia* (cf. above, p. 55, footnote) which, before the

proteosomes cannot be easily distinguished from small starch-granules (disorder having occurred by the pressing), it will be sufficient to keep the filaments for several hours in highly diluted solution of an aniline colour.

(1) It is hardly necessary to remark, that the granulations directly produced in living cells by highly dilute ammonia represent the same product, as that of the caffeine proteosomes treated with ammonia.

(2) A part of the absorbed ammonia, however, must have entered into still more intimate combination (cf. above, pp. 47 and 51.)

(3) The attempt, therefore, to find in this difference an argument against us, is a failure.

cells have died, causes a copious production of granulations which represent the *ammoniacal derivative* of the active albumin.[1]

We see, then, on the one hand, a close similarity in *chemical* behaviour between the *living matter* and the *labile reserve proteid*, and, on the other, the notable difference that the latter can combine with ammonia and reduce alkaline silver solutions, while the former cannot.[2] If we, however, admit, that the lability is considerably increased by the 'organisation' of active albumin into living matter, and that the most minute chemical attack upon living protoplasm can bring on death long before every molecule of it has had a chance to enter into proper reaction with the attacking substance, we can understand why certain reactions succeed with the non-organised albumin that do not succeed with the *organised* albumin.

It will be conceded that the evidence which the nature

(1) The fact that by absorption of ammonia the silver reducing property of the labile albumin is preserved can be made use of in such cases as that of the snowberry (*Symphoricarpus racemosus*) where the presence of reducing sugar would be an obstacle to making the silver test. If the fleshy tissue of unripe snowberries is treated, first with caffeïne, then with 0·1 % ammonia, and then washed with tepid water to remove every trace of sugar, we can get, here also, an intense blackening of the proteosomes by alkaline silver solution.

(2) *Bokorny* and myself in the beginning of our studies declared this silver reduction, observed first in *Spirogyra*, to be caused by the *living protoplasm* itself, because we then only paid attention to the silver reduction going on in *living protoplasm*, and not to that in the vacuole, and, like those authors who rejected the definition of living protoplasm as a varying *mixtum compositum*, entertained the opinion that *all albuminous matter present in the cytoplasm is also an essential part of it*. Hence, our declaration was justified at that stage of development of knowledge. Later on, however, we ourselves recognised that it is a *new kind of reserve proteid*, which causes the silver reduction, a proteid that can occur in the cytoplasm as well as in the cell sap.

of the case admits of is in favour of the assumption that the labile reserve albumin, discovered by *Th. Bokorny* and myself, corresponds closely to the *active albumin* foreseen by my theory. All reserve proteids that were known before that discovery, were what I designate as *passive* proteids. These compounds, whether they compose aleurone grains or proteid crystals,[1] or are merely dissolved albuminous matter, are in all probability various derivatives of the originally formed *active* albumin which has lost its labile groups in becoming them. An accumulation of acids or other compounds in the vacuole may contribute to the transformation from the labile to the stable modification of the reserve proteid in cells that are still alive.

To sum up, it may be convenient for the reader to have pointed out the most striking differences between active and passive albumin.

1. The capacity for water of imbibition is greater in the active than in the passive albumin, as is clearly indicated by the vacuolisation of the proteosomes during their coagulation.

2. Many of the stronger bases have the property of yielding insoluble combinations with the active but not with the passive albumin.

3. Weak organic bases, as caffeine or antipyrine, separate the active albumin in the original labile condition from its solution, yielding it as bright droplets (proteosomes), a behaviour not observed with passive albumin.

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(1) A phenomenon reported by *Peters* (Bot. Centrbl., 48, 181) is, in this connection, worthy of recording. This author observed in cells of *Carex* and *Sparganium* that the formation of proteid crystals commences in the interior of a *drop-like accumulation of albuminous matter*.

4. Alcohol of 10 per cent. ether vapour, and dilute prussic acid, coagulate the active, but not the passive albumin.

5. Active albumin reduces highly diluted alkaline silver solutions, the passive does not.

A few remarks may here he added in regard to *enzymes,* which we have to consider as bodies related, to a certain extent, to the proteids of the living protoplasm.[1] Both are changed by heat, acids, alkalies; but enzymes not so easily as protoplasm. Certain compounds, however, which kill the latter, as alcohols, will (in a short time) not change the former. Chloroform merely retards enzyme action (*Salkowski*). But, on the other hand, a considerable difference in resistance is found among the enzymes themselves. Diastase, *e.g.*, is killed by highly dilute hydrochloric acid, while pepsin is not. Prussic acid of 25 % will destroy in 12 hours the diastatic but not the proteolytic enzyme of the pancreas.[2] Certain bacterial enzymes are rendered inactive by hydrogen sulphide.[3]

That enzymes owe their activity[4] to certain labile atomic groups, has been surmised by a few authors, and

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(1) That enzymes belong to the class of albuminoid bodies has often been asserted and, again, often denied. I can draw, however, absolutely no other conclusion than that the enzymes of the pancreas which I have investigated (*Pflüg.* Arch., 27, 208) belong to the proteids; *Wurtz* proved this for papain; *Osborne* for the diastase of malt (18th Ann. Rep. Agr. Exp. Stat. of Conn.); *Seegen* and *Kratschmer* for the diastase of the liver (Jahresber. f. Thierchemie, 1877).

(2) *O. Loew, l.c.*, pag. 208; cf. also *Schär*, Jahresb. f. Thierchem., 1875, p. 269.

(3) *Fermi* and *Bernossi*, Arch. Hyg., 1894.

(4) The fact that different carbohydrates require different enzymes for their hydrolysis had long ago awakened the interest of physiologists, and I had again called attention to it, in *Pflüg.* Arch., 27, 210; the conclusion of *E. Fischer* that the configuration of the carboyhdrate must in a certain way

was recently again pointed but by *Ferdinand Hüppe*,[1] but the question as to the nature of the labile atomic groups is not yet satisfactorily settled. Aldehyde groups can, at least in the most common enzymes, only be present in a less reactive polymeric form, if at all; but the presence of highly labile amido-groups seems to be indicated by the noxious action of form-aldehyde upon them.[2] In general, the enzymes, although belonging to the labile proteids show a behaviour different from that of the 'active albumin' which *Bokorny* and I have found in plant cells, as they neither enter into reaction with organic bases, nor have action upon alkaline silver solution.[3]

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correspond to the configuration of the enzyme was therefore close at hand. It is the same principle as that I applied, nearly two years before *Fischer*, to explain the specific action of certain alkaloids upon the protoplasm of different cells and organisms. Cf. Ein Natürliches System der Giftwirkungen, p. 85, (1893).

(1) Ueber die Ursachen der Gärungen *etc.*, p. 35.

"Dass die labilen Eiweisskörper, gleichgültig ob sie von der Zelle trennbar sind oder nicht, aber ganz ausserordentliche Bewegungen ausführen und dadurch auch auslösen können, ist gerade durch die bacteriologischen Untersuchungen der letzten Jahre sichergestellt. Welche geringen Mengen Enzyme vermögen als Fermente hydrolytische Spaltungen oder Gerinnungen herbeizuführen! Wie geringe Giftmengen eiweissartiger Natur, Toxalbumine, genügen, um die Vergiftungen von Tetanus und Diphtherie herbeizuführen! Und wie energisch schützen die activen Eiweisskörper des Blutserums, natürlich immuner oder immunisirter Thiere das Thier gegen die eindringenden Parasiten und deren Gifte!"

(2) *O. Loew*, Natürl. System der Giftwirkungen, p. 71. The poison of the bacilli of tetanus (*Uschinsky*) and that of the micrococci of diphtheria (*Burckhard*) are also destroyed by dilute formaldehyde.

(3) *Nencki* and *Macfadyen* observed with one enzyme only, *viz.*, one of the cholera bacillus, reduction of an alkaline silver solution (1891), while *Brieger* obtained a phenylhydrazone with a proteid contained in a culture of the micrococci of diphtheria.

CHAPTER VI.

LIVING PROTOPLASM AND CHEMICAL LABILITY.

I have in Chapt. III drawn the inference that the proteids of living protoplasm are *different* from those of dead protoplasm; a new hypothesis as to the production of proteids, described in Chap. IV, led me further to a highly probable conception of the *nature* of that difference, while in the last chapter *facts* have been described, which prove the existence of a labile form of albumin. Let us now examine whether and in what degree the proteids composing the *living protoplasm* itself resemble the *labile* organic compounds of chemistry, and whether my view as to the *nature* of that lability can be supported by *facts*.[1]

A *labile* position exists if in a molecule one atom is influenced simultaneously by the affinities of two neighbouring atoms and thus becomes subject to an *oscillatory movement* and possessed, therefore, of *kinetic energy in the form of continuous atomic motion*.[2]

(1) There is some difference between the conception of *lability* and that of *instability*. Unstable compounds may merely contain much *potential energy* capable of being *suddenly* liberated and leading to explosion, as nitroglycerol, diazo-compounds, or fulminating silver. The other kind of instability, which is what we here have in view, consists in the presence of *kinetic energy* and leads not to explosion but merely to an *atomic* migration within the molecule, whereby *molecular* motion, *i.e.*, heat, is generated. A labile position of atoms bears to chemical affinity a relation similar to that of a mechanically unstable system to gravity.

(2) A remark of *Grant Allen* in his famous publication: "*Force and Energy*" is in this connection of interest: "Atomic motion may be

Such labile atoms, compared with all other atoms in the same molecule, occupy an exceptional position in this regard. We know that the atoms with their sphere of action occupy in different compounds different volumes; thus oxygen in the aldehyde group occupies a larger volume than in the hydroxyl group, the ratio being 1 : 0·6. The atomic volume of nitrogen in the cyanogen group is larger than in the nitro-group, and in this, again, larger than in the amido-group, the ratios being 1 : 0·50 : 0·13. And if the atomic volume of hydrogen were determined carefully in different compounds, we should very probably find, that it occupies, in an aldehydic and in an amido-group a larger volume than in a methyl and in a hydroxyl group.

Very justly, *F. Stohmann*, who has earned such great credit by his extensive calorimetric investigations, remarks: "The total energy of organic compounds consists of two parts; one part, the larger one, is determined by the number and kind of the atoms, the other, smaller, part is determined by the constitution and is therefore variable in isomeric compounds. This second part stands in close relation to the molecular volume, specific gravity, melting and boiling point, refractive power, and the degree of stability of the body." We may be justified in adding: the former and larger amount is *potential*, the latter and smaller part is, in many cases at least, *kinetic* energy.

Indeed, the greater the molecular volume of a compound compared with its isomers, the more kinetic energy it must

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separative as in decomposition, or aggregative as in the act of combining. The *continuous* or neutral stage is not at present known, though there is reason to think that it exists." Indeed, physicists have thus far paid but little attention to the considerable amount of energy present in labile compounds.

possess in form of atomic motion;[1] the more easily its transformation into a stable isomeric or polymeric compound will take place; and the larger will also be the amount of its *contraction*. Such transformations can bring on a very considerable change in the chemical character. I may mention, as examples; the transformation of ammonium isocyanate into urea, of hydrazobenzene into benzidine, of hydrobenzamide into amarine, of ketoximes into amides, of pinacone into pinacolin, and of maleïc acid into fumaric acid.

One of the most interesting labile atomic groups is the aldehyde group, $-C^{=O}_{-H}$, in which the oxygen exerts an attracting influence upon the hydrogen connected with the carbon atom, this being generally tetravalent, but sometimes only bivalent. The hydrogen atom is thus ever oscillating, between the carbon and the oxygen[2] as may be indicated by the following formulæ :—

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(1) We observe, *e.g.*, that ethylaldehyde has a larger specific volume than the less labile ethylene oxide, the ratio being 56·4: 50·6. Unsaturated carbon compounds occupy a larger molecular volume than that calculated from the data obtained from saturated ones. Further, the saturated hydrocarbons, alcohols, and acids of the methane series are, in comparison with the aldehydes, very *stable* compounds; so also are the saturated hydrocarbons of the benzene series. The stability, however, decreases with the number of entering hydroxyl groups. The stability of acids decreases also by the entrance of an amido-group, *e.g.*, amidomalonic acid gives off carbon dioxide at a much lower temperature than malonic acid. Besides, the *relative* position has an influence; β-amido-acids are more easily decomposed than α-amido-acids. Further, in fatty acids the hydrogen atoms in the α-position are more labile than the others and more readily replaced.—Certain labile compounds, again, show very great affinity for molecular oxygen, as zinc ethyl, dimethyl-arsine, monobrom-acetylene and the sodium compounds of ketones and aldehydes, all of which burst into flame in contact with air.

(2) I defined, as early as 1882, the lability of the aldehyde group as kinetic energy in form of atomic motion (Die chemische Kraftquelle im lebenden Protoplasma, p. 22).

$$-C\overset{=O}{\underset{-H}{}} \qquad -C^{-O-H} \qquad -C\overset{=O}{\underset{-H}{}} \qquad -C^{-O-H}$$

(1) (2) (3) (4)

These motions are accelerated by rise of temperature; in the same measure the inclination to chemical reactions increases. Ammonia, diamidogen, hydroxylamine, hydrocyanic acid, hydrogen sulphide, and primary sulphites, act with great facility upon aldehydes, and even molecular oxygen is taken up easily by many aldehydes. Certain substances bring on a rapid change: thus a little sulphuric acid will transform ethaldehyde into paraldehyde, whereby a contraction and development of heat takes place. Caustic potash converts this aldehyde into a resin.[1]

Another labile group is represented by the following position:—

$$\begin{array}{l} -C=O \\ \quad | \\ -CH_2 \end{array} \quad \text{, easily passing into :—} \quad \begin{array}{l} -C-OH \\ \quad \| \\ -CH \end{array}$$

In short, the lability of aldehydes and ketones can be considerably modified by the entrance of other groups. It can be increased by an amido-group and lessessed by a nitro-group, as the comparison of amidobenzaldehydes with nitrobenzaldehydes shows. Much depends, however, upon the relative position which the entering group occupies; further, alternating keto-groups bring on a greater lability than when they are immediately connected.

(1) The lower aldehydes in the methane series have more chemical energy than the higher members; the tendency to condensation and polymerisation, the action upon amines, *etc.*, decrease with the rise in the series. Aldehydes are, moreover, more energetic than the corresponding ketones; while, *e.g.*, ethaldehyde decomposes ammonium carbonate with lively effervescence, acetone has no such action.

(2) A certain degree of lability is, however, still observable in the ketone acids; thus, pyruvic acid is even more easily acted upon by hydrogen sulphide than acetone.

Let us now compare the principal characteristics of *labile compounds* with the behaviour of *living protoplasm* :—

1. Labile compounds are easily changed by heat, acids, or alkalies into stable, isomeric or polymeric products, whereby contraction takes place.[1]

Living protoplasm is also changed under such influences; it loses its chemical activity entirely and betrays its changed chemical character by being now very easily stained by even highly diluted solutions of aniline colours. Further, the *physical* characters of protoplasm are altered, the peculiarity of the osmotic membrane of the cytoplasm has given place to the properties of a filter, a change which can only be explained by a disruption of the organisation, in consequence of the contraction of the molecules of the plasma-proteids themselves, followed, in many cases (when death is not instantaneous), by a visible contraction of the entire cytoplasm.[2]

2. Labile compounds develop heat in their transforma-

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(1) In certain instances the labile modification can be easily got back from the stable one. Thus *Wislicenus* prepared two isomeric modifications of formyl-phenylacetic ether, the one solid and acid, the other liquid and neutral. The liquid modification passes into the solid one at very low temperatures, while the reverse takes place at the ordinary temperature. Caustic soda, also, transforms the liquid into the solid modification (Chem. Ztg., 1895. Nr. 78).

(2) Peculiar molecular motions, also, such as are exerted by alcohols, esters, chloroform, *etc.*, even when largely diluted, can change living into dead protoplasm. Here the toxical action of the alcohols of the methane series increases with the molecular weight. Unsaturated alcohols, however, have other, secondary, and more poisonous properties. Cf. the publications of *Meissner, Dogiel, Gibbs* and *Reichert, Mering, Tsukamoto*, and others.

The phenomena of disorganisation in protoplasm exposed to various noxious influences have recently been more minutely studied by *F. Klemm* (Jahrb. f. wiss. Bot., 28, 674).

tion into stable ones. Atoms, in such a state of continuous movement as prevents them from aggregating with neighbouring atoms, are in *equilibrium mobile.* If, now, this aggregation is accomplished by external influences, a stable position is established, the energy of *atomic* motion has passed into energy of *molecular* motion, *i.e.*, heat. Now, in regard to living protoplasm the measuring of small changes of temperature is only possible in higher animal organisms, for obvious reasons, and here, indeed, we observe *a rise of temperature,* when, soon after the death of the nervous system (which dies first), the *muscular tissue also succumbs,* being no longer nourished by currents of oxygenated blood. The long-known, so-called, *post mortem* rise of temperature finds thus a simple explanation. This phenomenon is accompanied by an increase of acid reaction and the setting in of stiffness ('*rigor mortis*').

3. Labile compounds can, by means of their containing kinetic energy, bring on chemical changes in other compounds. (*cf. Chap. VII.*)

4. Compounds containing labile hydrogen atoms are particularly inclined to attract the molecular oxygen of the air and in this respect present great analogy to living protoplasm. (*cf. Chap. VIII. p.* 108-110.)

5. Labile compounds enter readily into reaction with many other substances.

The potency of the vibrations of the labile atoms, facilitates chemical reactions. Must we not recognise analogy to the behaviour of living protoplasm when we see that numerous substances, even highly diluted and neutral, can attack protoplasm and kill it? A

minute specific attack, so insignificant that it is impossible to trace it chemically, can suffice to lead gradually to the collapse of the entire protoplasm of a cell, and if this cell happens to occupy an important position in a nerve centre, death may spread along the nerves like an irritation, and, since the action of the heart is then soon brought to an end, death of the muscles must follow. In this way, a minute quantity of a strychnine salt, or of prussic acid, or abrin can, as it is not retained by the blood or lymph, reach the nerve centres and bring on the death of myriads of cells that have not themselves absorbed a trace of it.

Living protoplasm behaves, indeed, like a most delicate indicator of the chemical character of a substance. Merely by the introduction of a carboxylic or of a sulpho group, *Nencki* converted strong poisons into innocuous compounds. On the other hand, a harmless body may become a poison, if an imido-group is formed by the migration of a hydrogen atom from carbon to nitrogen, as is the case with the non-poisonous hydrobenzamide, when it is converted into the isomeric amarine. It is obvious that a subtle reaction must have been made possible by the changed chemical character. The more labile compounds are, the quicker will they act upon each other, and the more can they remain active under high dilution. *Convinced of the logical necessity of the presence of labile atomic groups in the living protoplasm, I could not help seeing in the great proximity of aldehyde and amido-groups to each other, as indicated by my hypothesis of albumin formation, the very condition I had, long ago, endeavoured to make out.*[1]

(1) I had predicted in 1881 (*Pflüg. Arch.*, 25, 152) that amido-aldehydes, then an unknown class of bodies, would be prepared in the near future, and

Amido-aldehydes are of very great lability. Ortho-amido-benzaldehyde can exist in combination with hydrochloric acid in concentrated solution, while diluted acid changes it rapidly (*Friedlænder*). Those of the fatty acids, prepared by *Wolffenstein*, can reduce the metal, not only from gold, silver, and mercury salts, but also from copper solutions.[1] Amido-ethaldehyde is converted, soon after being set free from its combination with hydrochloric acid, into a gelatinous mass, and loses thereby its aldehyde character (*E. Fischer*). Diamido-acetone shows the same behaviour, passing into an amorphous compound of totally different properties.[2] Compared with amido-aldehydes, many oxy-aldehydes, such as the aldose-sugars, appear as relatively stable compounds.

The question now arises: can it be *proved*, directly or indirectly, that aldehydic and amido-groups are really present in the proteids of the living protoplasm?—If our hypothesis is correct, that vital motion emanates from these groups, it follows, that every substance, acting in great dilution upon them, must prove a *poison for every living vegetal or animal organism*, because the chemical character of *living* matter is thereby changed. Observation confirms this inference. Aldehydes are very easily acted upon by hydroxylamine, prussic acid, hydrogen sulphide, sulphurous acid (acid sulphites), diamidogen,

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would prove to be much more easily changeable than ordinary aldehydes. Soon afterwards, *Paul Friedlænder* made us acquainted with ortho-amido-benzaldehyde and, later on, *Wolffenstein* with the first amido-aldehyde of the fatty series, thus proving the correctness of my predictions. The latter obtained such aldehydes by careful oxidation of piperidine, coniine, and β-pipecoline with peroxide of hydrogen.

(1) Ber. D. Chem. Ges., 25, 2777 and 28, 1461.

(2) *Rügheimer* and Mieschel, ibid., 25, 1563.

phenylhydrazine, and amidophenol, while labile amido-groups enter very easily into reaction with free cyanogen, nitrous acid, and form-aldehyde. *Now all these compounds are also strong poisons.*

What an enormous change in poisonous properties is observed, when one hydrogen atom of the ammonia-molecule is replaced by the hydroxyl, or by the amido-group! While ammonia in form of neutral salts is only a weak poison for animals, the substitution-products thus obtained, viz., hydroxylamine and diamidogen are, in the same dilution and in *neutral solutions*, very strong poisons. Bacteria and mould fungi, that are not poisoned by concentrated solutions of neutral ammonium salts, are easily attacked by hydroxylamine, even at a dilution of 1 : 10,000.[1] *Marpmann* observed, later, its poisonous effect also upon pathogenic microbes. I have found, that diatoms are killed within 24 hours by hydroxylamine in a dilution of 1 : 100,000, and phænogams in a dilution of 1 : 15,000, in a few days.

While ammonia is, next to nitrates, the most important source of nitrogen for the nutrition of plants, hydroxylamine never can be used for this purpose, being a deadly poison!

In a dilution of 1 : 20,000 it is a stronger poison for infusoria than strychnine; in 1 : 10,000 it kills crustaceans within three hours; in 1 : 20,000 various aquatic animals within 36 hours (*Loew*); 0·05 g of the hydrochloride kills a pigeon in three minutes; 0·1 g pro kilo of a warm-blooded animal will produce convulsions (*Raimundi* and *Bertoni*; *Binz*; *Lewin*).

(1) *O. Loew*, Pflüg. Arch., vol. 35, p. 515 (1885).

When diamidogen was discovered by *Th. Curtius*, in the year 1887, and its remarkable affinities for aldehydes became known,[1] I predicted at once, that it would prove a to be a universal poison, a poison for all kinds of living cells. In fact I have observed that the neutralised sulphate in a dilution of 1 : 10,000 kills algæ in from one to two days; further, in less than a day at 1: 5000, bacteria; and at 1 : 2000, infusoria, crustacea, mollusca, and aquatic larvæ of insects, while neutral ammonium sulphate in this dilution produces not the slightest effect whatever. I have found, further, that young sun-flower plants die within 4 days, and barley within 2 days, when placed in a solution containing 0·2 per mille of neutralised sulphate of diamidogen while the same plants treated with equal quantities of ammonium sulphate developed normally.[2] 0·5 g. of diamidogen sulphate will kill a rabbit in less than two hours (*Buchner*).

How is it that hydroxylamine and diamidogen are such strong poisons compared with ammonia? The answer can only be found in their marked difference of behaviour to *aldehydes*. It has been demonstrated by recent investigations, that salts of hydroxylamine and of diamidogen act even in high dilutions upon aldehydes, while ammonia acts only either in the free state or as carbonate and with less energy. This difference is due to the *greater lability of the hydrogen atoms* in the former enabling them to attack more easily the labile oxygen of aldehydes.[3]

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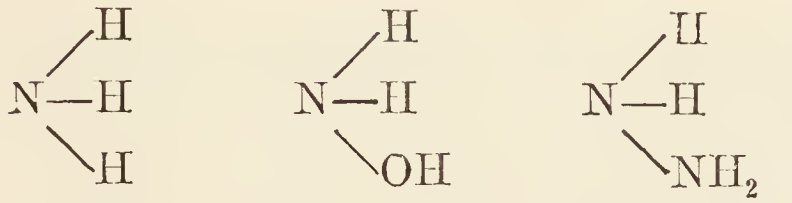
(1) According to *Curtius*, diamidogen attacks *aldehydes* even in strong acid solutions, while it combines with ketones only when in the free state.

(2) *O. Loew*, Berichte d. D. Chem. Ges., vol. 23, p. 3201.

(3) The reaction upon ketones is much less energetic.



Ammonia. Hydroxylamine Diamidogen.

In further perfect accordance with the aldehyde theory, I have found aniline to be a weaker poison than phenylhydrazine or amidophenol ; phenol to be a weaker poison than amido-phenol; and pyridine a weaker poison than piperidine.

It is of considerable interest, that *certain derivatives of hydroxylamine* also react easily with *aldehydes* and exhibit accordingly a *poisonous character* ; these derivatives are the 'amidoximes,' prepared by *Tiemann* by the action of concentrated hydroxylamine upon nitriles at higher temperatures. Of benzenyl-amidoxime, $C_6H_5\,C\langle^{NH_2}_{NOH}$, 0·1 gr. suffices to kill a rabbit.[1]

Again, prussic acid is a poison for all kinds of living organisms, but it attacks lower organisms less energetically than the higher ones.

In a dilution of 1 : 2000, it kills the lower aquatic animals within a few hours, and, in dilution of 1 : 1000, phænogams, algæ, and the lower fungi, after about 15 hours.

While *prussic acid* acts easily upon the *aldehyde* groups, free *cyanogen* attacks readily labile *amido* groups. Accordingly, the latter forms just as general a poison as the former and, for the *lower* organisms, even a more violent one. In dilution of 1 : 5000, it kills bacteria and

(1) *Mering*, Ber. D. Chem. Ges., 1885, p. 1045. An exceedingly poisonous compound is also phenyl-hydroxylamine, discovered by *E. Bamberger*; cf. Berl. Klin. Wochenschrift, 1895, 41–42.

yeast cells, of 1 : 10,000, algæ, phænogams, and lower aquatic animals[1] ; 0·02 gr. suffices to kill a cat (*Bunge*), for which only 0·004 gr. of prussic acid is enough.

Of special interest in connection with the present subject is the highly poisonous character of *nitrous acid*, compared with other mineral acids, a fact, again easily accounted for by my theory, since it acts as an energetic oxidising agent upon *amido*-groups. Free nitrous acid in a dilution of 1 : 100,000 kills algæ within 48 hours.[2] Nitrites are poisonous in all those cases, where nitrous acid is set free from them by the organism ; therefore, for plants containing free acids or acid salts. To the higher animals also nitrites are very noxious, as the observations of *Binz*, *Emmerich* and *Tsuboi*, and others have shown.

Then, too, the poisonous action of *form-aldehyde* only becomes intelligible by taking into consideration its readiness in affecting *amido*-groups of a certain lability: in a dilution of 1 : 10,000 it kills microbes and algæ ; while in solution of 1 : 2000 it kills, in 2 hours, crustaceans, worms and mollusca.[3] Rabbits are killed by 0·24 gr. pro kilo. (*Zuntz*). Phænogams, watered with a 1 p. m. solution, die in from two to six days (*Bokorny*).

On contemplating these numerous toxicological facts, it will be admitted that there exists perfect accordance between the anticipated phenomena and the actual ones, *i.e.* the proteids of *living* protoplasm have an aldehyde character, while those of *dead* protoplasm have no

(1) *O. Loew* and *M. Tsukamoto*, Bulletin of the Agricultural College of the Imperial University, Japan. II. No. 1.

(2) *O. Loew* and *Th. Bokorny*, Botan. Ztg., Dec. 1887.

(3) *O. Loew*, Jahresb. f. Thierchemie, 1888.

such character; the former have affiinity for hydroxylamine, diamidogen, or prussic acid, the latter have not. The views of *Pflüger* and of *Latham*, where it is assumed that the labile groups are cyanogen groups, indicate a different behaviour.[1] Moreover, according to the view of *Pflüger*, the change connected with loss of life consists in the chemical *fixation of the elements of water*; according to my view, it consists in a *migration of atoms* from labile to stable positions, without water being taken up.

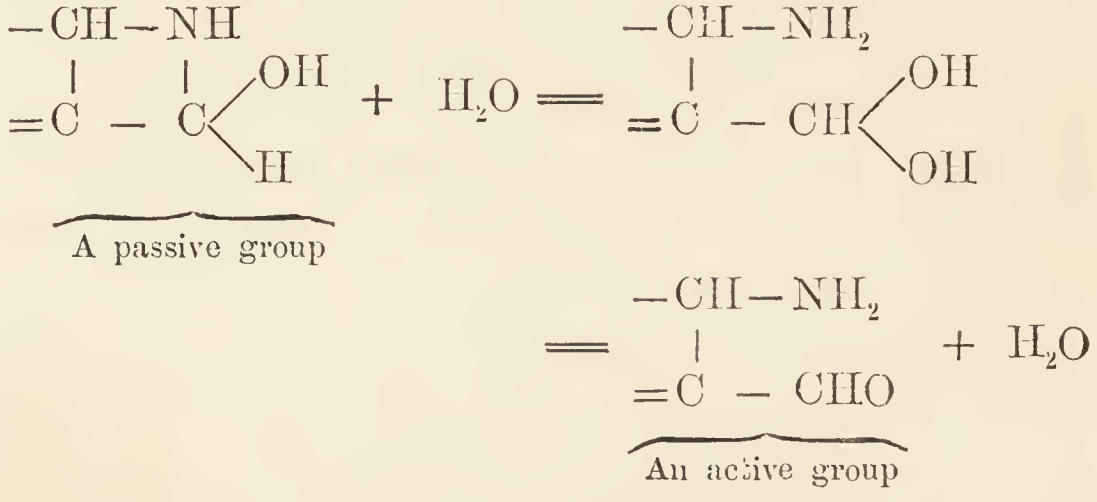
As my deduction is verified by facts, the further inference may be drawn that "the more labile the aldehydic or amido-groups contained in a compound, the stronger is its poisonous property." But as labile amido-groups act readily upon labile aldehyde groups, there constantly exists the danger to the living cells themselves of an inter-molecular poisoning taking place; indeed one needs only heat the living organisms to 50° for death to result, with rare exceptions; the threatened danger has become a reality.

The easy change from the living to the dead state must, however, on the other hand, lead to the inference that energy has to be *spent* by living cells in transforming *passive* proteids into *living* protoplasm, a conclusion, *Pflüger* arrived at in the year 1875.[2] Here kinetic energy, as such, is taken up into the molecule. The regeneration of the labile groups in the transition from

(1) Hydroxylamine will act upon *cyanides* (nitriles) in concentrated solutions and at higher temperatures; upon *aldehydes*, even in the cold and in high dilution. Prussic acid is without action upon nitriles.

(2) *Pflüg.* Arch., 10, 308. "Bei der Gewebebildung wird eine Arbeit geleistet, wodurch die Cohæsion des Eiweissmoleculs ausserordentlich gelockert erscheint."

passive into active albumin[1] could, in the sense of the theory developed in Chap. IV, be represented in the following manner, water being taken up and yielded again:



In scrutinising the general behaviour of protoplasm, we have to take one step farther, and to infer that not only is the *chemical* structure of the proteids a labile one, but also the *morphological* structure of the protoplasm that is, the *tectonic*, the invisible arrangement of the molecules of active albumin into small particles of protoplasm. Not only *slight* disturbances of a *chemical* nature, but those also of a *mechanical* nature in some *minute* portion of a living cell, may cause the collapse of the entire protoplasm. *Morphological* structure and *chemical* nature are evidently most intimately connected; an injury to one will also damage the other: *Living protoplasm is a labile structure built up of labile material.*

Naturally, there exist various degrees of lability in different kinds of protoplasm. The power of resistance to noxious influences varies not only with the lower or

(1) In plants this transformation, when connected with transportation, of proteid, takes a more indirect way, *via* amido-compounds; cf. Chap. IV.

higher development of different organisms, but also with the nature of the different cell-groups of one and the same organism ; it varies even with the organoids of a cell, for the nucleus and chloroplasts of plant-cells are much more sensitive to many noxious influences than the cytoplasm.

Those cells or organs which have the most delicate office to perform are the very first to perish. The more labile the tectonic of a protoplasm the greater its efficiency will be and the more dangerous a slight injury will prove to it.

Further, the labile character of protoplasm is the essential condition for its *irritability*. Only labile compounds exhibit properties which have a resemblance—although a remote one—to the phenomenon of irritability, for these only may, by seemingly slight influences, be changed into isomeric or polymeric stable compounds. Thus, *e.g.*, rubbing with a glass rod can transform the labile benzophenone (melting point 27°C) into its stable isomer (melting point 49 °C). A trace of triethyl-phosphine will transform methyl-isocyanate into a polymeric form, with liberation of heat (*Hofmann*).[1]

Now, when any irritation of the living protoplasm takes place, *chemical change of the living matter* is excluded, for that would mean death; protoplasm can supply the energy required merely by retardation of its usual atomic motions. The heat of respiration can quickly cover the loss

(1) A still *more remote* example of quick change by an apparently insignificant influence is exhibited by Glauber's salt, in supersaturated solution. In this, a *trace of dust* on a glass rod, with which it is touched, may bring on crystallisation with development of heat, after which the stable condition may easily be reconverted to the unstable one by applying heat. I here avoid, for good reasons, citing such cases as examples as lead to a perfect destruction of the compound.

incurred, by passing into atomic motion. I cannot accept the opinion of *Haeckel*, when he speaks of 'souls' in atoms, and the perfection of these souls in the living protoplasm;[1] and cannot agree with *Nägeli's* inference that "because sensibility is a property of albumin molecules, we must ascribe it also to other compounds."[2] Clearly, it is a erroneous supposition that *ordinary* albumin molecules have *sensibility*; this property stands and falls with *lability* and proper organisation (tectonic). Not with matter itself, but with *lability* of matter, *irritability* is possible; not in the atoms themselves but in the labile position of atoms in proteids does the first trace of vital energy manifest itself.

(1) Tageblatt der Naturforscherversammlung zu München, 1877. p. 22.

(2) Ibid. p. 39.

CHAPTER VII.

THE CHEMICAL ACTIVITY OF LIVING CELLS.

The great chemical activity of living cells astonishes the pondering mind. Constructive, as well as destructive metabolism, betrays the presence of a power admirably adapted to do chemical work. The synthesis of carbohydrates, the transformation of starch into cellulose and fat, the production of the highly complicated proteids, are executed with great readiness by plant-cells. Animal protoplasm, also—although, in extent and power of chemical synthesis, far excelled by vegetal protoplasm—is capable of many highly interesting chemical operations, such as the construction of living protoplasm out of inert albuminous matter, the formation of hæmoglobin, mucin, elastin, keratin, and collagene from the proteids of the food, the production of enzymes, or the transformation of sugar into fat. Not less remarkable than the synthetic work is the energetic oxidation exhibited by the respiration process.[1]

The character of the chemical work in plant-cells is many-sided: polymerisation, condensation, esterification, formation of amido-compounds, generation of the cyclic constitution, reductions, and oxidations are included in it. We find acids and bases, aldehydes and ketones, ethers, alcohols, and sulphides in the vegetal kingdom; but

(1) See the following chapter.

neither aldoximes, nor ketoximes,[1] neither sulpho-acids, nor nitro-, nor azo-compounds. Thus, the chemical activity, varied as it is, seems, nevertheless, not to leave certain channels.[2] A number of chemical operations can be carried out by every plant-cell; others only by specifically endowed ones; and, while certain compounds are found in all cells, such as carbohydrates, fats, and proteids, others are not of universal occurrence, though still very frequent, such as tartrates, succinates, glucosides, resins, tannins. Others, again, are very rare, being either restricted to one family, as quinine or strychnine, or to a few families, as caffeïne.

In scrutinising the chemical activity of a plant-cell, we have at first to pay attention to the differentiation of the plasmic contents. The cortical layer of the *cytoplasm* has not the same function as the inner layer or *tonoplast*; the former produces the cellulose-wall from starch or sugar while the latter restrains noxious substances accumulated in the vacuole from returning into the organoids. The *leukoplasts* form starch from sugar, thereby preventing a higher concentration of the sugar-solution, which would, to some extent, check plasmic activity. The *chloroplasts*, again, with the aid of ethereal oscillations of a determined

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(1) No derivative of hydroxylamine has, as yet, been discovered in organisms.

(2) It is a notable fact, for which explanation has, hitherto, not been given, that especially such benzene derivatives as have a *lateral group of three carbon atoms* frequently occur in plants, some in the form of glucosides. I may mention: hesperidin, caffeic acid, cumarin, umbelliferone, daphnetin, coniferyl-alcohol, eugenol, anethol, safrol, cinnamic alcohol and aldehyde, cinnamic acid, thymol, scopoletin, asarone, syringin, æsculetin, absinthol, camphor, the terpenes. Also, tyrosine and phenylamido-propionic acid, as decomposition-products of proteids, have to be mentioned.

wave-length, transform carbonic acid into sugar.[1] The *nucleus*, finally, is charged, among other things, with the duty of producing enzymes.

A plant cell deprived of the nucleus is incapable of forming cellulose (*Klebs*); very probably on account of suspension of the production of diastase necessary for a sufficient saccharification of starch for that process. For the lowest *animal* forms, an analogous case was proved by *B. Hofer*; amœbæ can no longer digest albuminous particles when deprived of their nucleus.[2]

That the vegetal nucleus seems to be the manufacturer of the proteids, was first suggested by *Strassburger* and *Schmitz*; proteid-crystalloids are frequently found in the nuclei of plant-cells, especially in the orders, *Oleaceæ*, *Scrophularineæ*, *Bignoniaceæ*, and in the *Pteridophytes* (*Zimmermann*). It does not seem probable that this proteid has its source in the cytoplasm and is then transported into the nucleus for crystallisation. Again, in germinating seeds, where proteid formation from fragments of decomposed reserve-proteids proceeds with considerable activity, the nuclei (and also the nucleoli) increase in size.[3]

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(1) In the assimilation of carbonic acid it is generally assumed that formaldehyde is the first product. This yields, however, upon condensation in solution, as I have shown, not dextrose but other sugars; moreover, it is poisonous. Therefore, I have added the hypothesis that the formaldehyde combines at once with certain hydroxyl-groups in the protoplasm of the chloroplasts, *the amido-groups being protected*. The condensation taking place afterwards must thus lead always to one and the same configuration of the resulting sugar, since the molecules of form-aldehyde have lost their freedom of motion. Cf. *O. Loew*, Ber. D. Chem. Ges., 1889, pp. 484 and 473.

(2) Further observations in this regard were made by *Korschelt* and by *Verworn* (*Pflüg.* Arch., 51).

(3) *Peters*, Botan. Centralbl., 48, 181. Also in the cells of glands relatively *large* nuclei are often present. It is also a remarkable observation that plant-cells deprived of their nucleus do not produce

The nucleus plays also a certain part in the *organisation* of the cytoplasm. In cases of mutilation, only such cells can be regenerated as still contain the nucleus (*Nussbaum, Gruber*). Since the *growth* of vegetal cells bears a certain relation to the nucleus, as was observed by *Klebs* and *Haberlandt*,[1] it is probable that the *nucleus* prepares the proteids suitable for the purpose of organisation. The vegetal nucleus performs this by far-reaching synthesis, the animal nucleus by transforming dissolved proteid[2].

The most important function of the nucleus, however, is connected with the division and multiplication of cells and with sexual propagation in plants and animals. The nuclein known to chemists, extracted with alkaline liquids

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any more starch by assimilation. Only certain algæ, containing pyrenoids (*Zygnemaceæ*) have thus far been observed to be exceptions in regard to starch-production under this condition (*Klebs*).— Simpler organisms, like amœbæ and algæ, are especially well suited for experiments with *isolated* cytoplasm. *Klebs* has, by plasmolysis, and *Gerasimoff*, by low temperature during the process of karyokinesis of algæ, obtained living cytoplasm *without a nucleus*. The life of such cytoplasm, however, did not last longer than about six weeks. On the other hand the nuclei of radiolaria can remain alive, if *deprived* of the *cytoplasm*, only 10-15 hours. Nucleus and cytoplasm influence each other by certain of their products (*Verworn, Pflüg.* Arch., 51, 113).

(1) Biol. Centralbl., 8, 133.

(2) I consider the process of organisation as a sort of polymerisation, in which only molecules of *the same configuration* can participate, and in which isomeric molecules would be hurtful, as the mutual reaction between their labile groups would be facilitated (Cf. *O. Loew*, Natural System of Poisonous Actions, Chap. V. On the *poisonous proteids*, 82). This, again, would imply a specific tectonic for the nuclei of different species as the necessary condition for yielding one and the same kind of active albumin for the organisation of each cytoplasm. The possible isomers and especially stereo-isomers of a proteid reach evidently to an immense number, even if we start from one and the same active peptone. Differences have been observed between the oxyhæmoglobins, fibrins and alexins of different animals.

and precipitated with acids, is a relatively stable compound,[1] which would be entirely incapable of serving the physiological phenomena of karyokinesis. These clearly point to a highly *labile* state of the albumin contained in nuclein[2].

Division of chemical labour, still of restricted compass in a single plant-cell, keeps growing in importance and extent with the development of multicellular organisms, both, in plants and animals.

The *glands*, (chemical factories in the service of an organism),—of simple structure in plants, but forming complicated organs in the higher animals,—betray again a far-reaching differentiation. They may secrete enzymes, carbohydrates, acids, fat, or wax. Products secreted by *vegetal* glands alone are terpenes and resins.[3] Glands secreting poisonous compounds (toxalbumins, etc.) occur in the mouth of snakes, in the skin of the toad, on the feet of scolopendras. Crustaceans and beetles produce chitin; spiders and caterpillars, fibroin; carabidæ, butyric acid; bees and ants, formic acid. How different again the products

(1) *Liebermann* showed that nuclein contains *metaphosphoric* acid, and *Kossel*, that bases of the xanthine series and nucleic acid are contained in it. My own observations make it highly probable that the nuclein of plants (the lowest algæ and fungi excepted) is present as a *lime* compound. Cf. *O. Loew*, On the functions of calcium and magnesium salts in plants, Flora, 1892, 368; Landw. Versuchs-Stat., 41, 467; Bot. Centrbl., 1895, Nr. 52. Also *Molisch*, ibid. Nr. 4.

(2) Differences have been shown to exist between the nuclei of nerves, glands, and muscles; further, between those of the male and female sexual cells, the former being richer in nuclein (*E. Zaccharias*). Still greater must be the differences between the nuclei of the germ-cells and those of the somatic cells, especially in animals.

(3) An elaborate treatise on the resin-producing glands of the conifers was recently published by *H. Mayr*, formerly professor in Tōkyō.

of the anal glands of the skunk, musk deer, beaver, and civet. Finally, the chemical labour of the liver varies with the different classes of animals. Dogs can produce ethyl sulphide (*Abel*) and kynurenic acid ; birds, ornithuric acid ;[1] the bile of geese contains chenotaurocholic acid ; that of swine, hyotaurocholic acid. Birds and snakes produce more uric acid than urea, while the reverse is the case of mammalia[2].

This very brief survey bears sufficient testimony to the extraordinary ability of living cells to do a great variety of chemical work ; such admirable chemical transmutations could not fail to arouse the lively interest of chemists and to instigate them to synthetic operations. Indeed, many organic substances have been artificially prepared, since the start was made by *Woehler* in the year 1828. Carbon and hydrogen were united in the electric arc by *Berthelot* ; acetylene, thus formed, leads, among other things, to ethylene, ethyl alcohol, acetic acid, aldehyde, acetone, and glyoxal. Acetylene yields at low red heat benzene, from which, again, numerous derivatives can be obtained. Acetone, further, may easily be converted into trimethyl-benzene, aldehyde into trimethyl-pyridine ; from pyridine, again, coniine may be reached (*Ladenburg*). Glyoxal leads, by way of its cyanhydrin, to tartaric acid.

Carbon can be united with metals and some of such products can yield on decomposition gaseous, liquid, and solid hydrocarbons. Methane, again, yields, methyl alcohol and form-aldehyde, while the latter yields

(1) This acid, a dibenzoyl-ornithine, is secreted after ingestion of benzoic acid ; ornithine again is probably a diamido-valeric acid (*Jaffé.*)

(2) Also, pathologic processes vary in animals; thus, phloridzin will produce diabetes in dogs, but not in rabbits or frogs.

by condensation several kinds of sugars (*O. Loew*). Formic acid can be obtained by the action of sodium upon moist carbon dioxide (*H. Kolbe*), or by the action of iron filings upon carbon bisulphide in presence of water in sealed tubes at 100° (*O. Loew*). Oxalic acid results on passing dry carbon dioxide over hot sodium amalgam (*E. Drechsel*), or on treating the product of reduction of carbon bisulphide by sodium amalgam with fusing potash or boiling baryta-water (*O. Loew*). From a mixture of oxalic ether and acetic ether, aconitic acid can be obtained (*L. Claisen* and *E. Hori*); from acetone and oxalic ether, oxytoluic acid. and from this, an anthracene derivative, (dimethyl-anthrarufin) has been obtained (*Claisen*). From malonic ether phloroglucin can be reached (*A. Baeyer*), and also caffeine (*E. Fischer*); from succinic ether, hydroquinol; from malic acid, oxynicotinic acid and daphnetin (*Pechmann*).

These, and similar synthetic processes, are, however, for the most part only possible by the application of powerful agents, such as strong bases or acids, sodium ethoxide, sodium amalgam, zinc chloride, etc., and partly by the aid of high temperatures; while *no light is thrown upon the special method followed by the living protoplasm* of plant-cells, a material consisting of proteids of neutral reaction, or nearly so, and of a very subtle nature forbidding a simple analogy with the ordinary chemical processes. We are led to assume an energy differing to some extent from the common chemical energy and consisting in *wave motions of a specific character* Attempts to explain the chemical actions of protoplasm by a kind of motion were first made, years ago, *e.g.* by *Nägeli*, in defining the fermentative activity of yeast. *Mc. Laughlin* went still farther, applying the laws of

oscillations established by physicists to the action of bacteria in infectious diseases.[1] But neither *Nägeli* nor *Mc. Laughlin* touched the question, *why such energetic motions stop at once on the death of a cell*, although the conclusion that the *living* protoplasm must consist of easily changeable, labile proteids was close enough at hand. *Mc. Laughlin* expresses his views upon the fermentative action of bacteria and yeast-cells in the following words: "The distinctive energy or waves of a cell, say a yeast-cell, can influence those substances only, whose waves bear a certain relationship to those of a yeast-cell; and they must be equal in their periods, direction, and, perhaps, in other characteristics, before those on one side can influence those on the other. The nature of this influence will again depend on whether the two sets of waves coïncide in trough and crest. If they do, the waves will supplement each other and their amplitude will be enlarged; if they do not, they will antagonise each other, and their amplitude will be diminished; or, it may be, the waves will be destroyed by mutual antagonism; it will be remembered that all this occurs in waves of sound, of light, and of water, and, if analogy has any merit, it can occur in waves of molecular energy."

This deduction, however, notwithstanding its scientific appearance, is not justified, as such *molecular* motions ought to be present also in *dead* cells. There exist evidently motions in the *living* protoplasm, but they are *much more powerful* than the atomic or molecular motions that may be connected with nutrients.

We are acquainted with chemical actions caused by

(1) Fermentation, Infection, and Immunity; 1892.

waves of heat, light,[1] electricity,[2] and even of sound (explosion of nitrogen iodide), but concerning the related phenomenon of *chemical action set up by oscillating motion of labile atoms*, our knowledge is but very scanty. Such processes are of the kind termed *katalytic actions*.[3] This expression was first used by *Berzelius* to designate chemical phenomena apparently caused by mere contact with a certain substance. The unsatisfactory definition of *Berzelius*, and the denomination of certain processes as katalytic which in reality were not such at all, implied a misunderstanding, and after *Liebig* had ridiculed the idea of *Berzelius*, this interesting group of phenomena was ignored for many years.[4]

Katalytic actions exist, however, but they are not the result of a mere contact, as *Berzelius* believed, but of a

(1) The action of *light* may consist in bringing about (*a*) *combinations*, as that between chlorine and hydrogen, or (*b*) *disruptions*; bisulphide of carbon is split into sulphur and a lower sulphide (*O. Loew*); aqueous sulphurous acid is split into sulphur and sulphuric acid (*O. Loew*); nitric acid into oxygen and nitrous acid; silver salts are reduced to metal; (*c*) *reductions* of organic compounds: in *alcoholic* solution quinone is changed into hydroquinol, nitrobenzene into aniline (*Ciamician* and *Silber*), benzil into benzil-benzoin, a portion of the alcohol present being converted into aldehyde; (*d*) *polymerisation* and *condensation*; thymoquinone, anethol, phenyl-naphthoquinone are polymerised; mono-bromacetylene is converted into tribrombenzene; propargylic acid into trimesic acid (Ber. D. Chem. Ges. 27, 958).

(2) Highly interesting syntheses have been accomplished by means of electricity by *Crum Brown* and by *W.* v. *Miller*.

(3) The great resemblance of the chemical activity of living cells to katalytic actions was recognised more than thirty years ago by the great physiologist, *C. Ludwig*. Also *C. Lehmann* in his "Lehrb. d. physiolog. Chem.," and, later on, *Traube* and *Stohmann* expressed themselves in the same sense.

(4) Even now-a-days voices are raised in the sense of *Liebig*. See *H. Macleod's* lecture at the meeting of the *British Assoc.* in *Edinburgh*, 1892.

certain amount of energy being conveyed, whereby the *katalyser* remains chemically *intact.*[1] If, however, the *apparently* katalytically acting substance undergoes intermediary chemical changes with final regeneration, the process is not a katalytic one. Such *pseudo-katalytic* actions are, for example, the part nitric oxide plays in the manufacture of sulphuric acid, that of cobaltic oxide in the development of oxygen from chloride of lime, the action of aluminium chloride in the synthesis of hydrocarbons, that of zinc chloride in the transformation of glycol into aldehyde, and the accelerating action of iron and copper salts upon the oxidation of phenol by peroxide of hydrogen.[2]

The genuine katalytic actions[3] may be divided into three groups :

1. Katalysis by labile organic compounds ;

2. Katalysis by mineral acids, alkalis, and certain salts ;

3. Katalysis by finely divided metals.

As examples of the first group may be mentioned the

(1) I cannot agree with *Ostwald*, when he says (Anla, 1895) : " Worauf die Wirkung der katalytischen Stoffe beruht, ist zur Zeit noch ein Rätsel, dessen Lösung um so schwieriger ist, als sie nur auf Grund *neuer Prinzipien*, welche über das *Energiegesetz hinausgehen*, gefunden werden könnte."

(2) Chem. Centralbl. 1885, p. 224. In certain cases the *apparently* katalytically acting substance is mainly a suitable medium to bring two compounds into more intimate contact with each other, or by combining with one of two compounds present to loosen somewhat certain affinities in the molecule, and thereby bring about combination with the second compound.

(3) *Stohmann* (Zeitschr. Biol. 1895 p. 389) defines katalysis as a " mode of atomic motion in molecules of labile structure which is brought on by an energy exerted from another body, and leads with loss of energy to the formation of more stable compounds."

conversion of free cyanogen into oxamide by a dilute solution of ethyl aldehyde, a reaction observed by *Liebig*; the transformation of thio-urea into the isomeric ammonium thiocyanate by an alcoholic solution of ethyl nitrite (*Claus*); the facilitating action of acetic ether upon the combination of hydrocyanic with hydrochloric acid (*Claisen* and *Mathews*). Maleïc acid transforms ketazines into the isomeric pyrazolines with liberation of heat,[1] whilst fumaric acid can only accomplish this at a temperature of 100°.

No doubt, the action of the enzymes also belongs to this group (cf. Chap. VI).

In the second group of katalytic actions may be counted the transformation of maleïc into the isomeric fumaric acid by mineral acids; of maleïc ether into fumaric ether by contact with hydrochloric acid at the ordinary temperature (*Skraup*) and of citraconic into mesaconic acid (*Delisle, Franz*); of hydromellitic into isohydromellitic acid by hydrochloric acid (*Baeyer*); of dihydroterephtalic into an isomeric acid by a solution of caustic soda; the formation of para-amidophenol from phenyl-hydroxylamine (*E. Bamberger*) and that of paranitroso-compounds from aromatic nitrosamines. Also, the transformation of oleïc into elaïdic acid by nitrous acid deserves mentioning.

In connection with the third group the following actions deserve attention: platinum black brings about an oxidation of hydrogen, of ammonia, of alcohols, and of various other compounds; it unites sulphur dioxide with dry oxygen to form sulphur trioxide; it combines hydro-

(1) *Curtius* and *Foersterling*, Ber. D. Chem. Ges., 27, 770. Maleic acid yields 326·3 units of heat, fumaric acid only 319·7 pro gram-molecule (*Stohmann*). Cf. p. 64.

gen with hydrocyanic acid into methylamine at 110° (*Debus*); it transforms a mixture of nitric oxide and hydrogen into ammonia and water; it accelerates the decomposition of hydroxylamine in presence of caustic potash; it decomposes peroxide of hydrogen energetically; it transforms ozone into common oxygen (*Mulder*); it decomposes azoimide and water into ammonia and nitrous oxide (*O. Loew*), and nitrososulphate into sulphates and nitrous oxide (*Pelouze*).

Finely divided iridium or rhodium decomposes formic acid into carbon dioxide and hydrogen (*Deville* and *Debray*); palladium powder effects the oxidation of hypophosphorous acid with liberation of hydrogen;[1] finely divided copper incites a rapid decomposition of formaldehyde by caustic potash with liberation of hydrogen,[2] it also decomposes diazobenzene chloride into nitrogen and chlorbenzene at low temperatures.[3] Zinc filings at 100° condense acet-aldehyde to aldol and croton-aldehyde.

All these actions of finely divided metals can be best explained by the assumption that a *modification of heat waves* takes place in such a manner that this energy can now pass more easily *into chemical energy*. With platinum black this modification is carried still farther by the dense layer of *oxygen* surrounding the metallic particles.[4]

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(1) *Engel*, Compt. rend., 110, 786. The chemical explanation given by this author is certainly incorrect.

(2) *O. Loew*, Ber. D. Chem. Ges., 20, 145.

(3) *Gattermann*, ibid. 23, 1218 and 25, 1091, footnote.

(4) Platinum-black can absorb 800 times its own volume of oxygen (*Doebereiner*); *Mond*, *Ramsay*, and *Shields* calculate much less; the kind of preparation may have some influence. But this *alone* would not suffice to explain its oxidising power, compressed oxygen possessing no increased energy at the ordinary temperature. Cf. also Ber. D. Chem. Ges. 23, 290 and 677, where I first gave the above explanation of the katalytic action of platinum black.

The katalytic powers of platinum-black are clearly of a very inferior character, compared with the faculties of living protoplasm,—but in regard to simpler reactions a parallelism can nevertheless be shown to exist, of which I here adduce some further proof. One of the most general synthetic processes is the formation of fat from glucose in living cells, a process consisting in condensation and reduction :

$$3C_6H_{12}O_6 - 16\ O = \underbrace{C_{18}H_{36}O_2}_{\text{(stearic acid)}}$$

$$C_6H_{12}O_6 + 4H = 2\ \underbrace{C_3H_8O_3}_{\text{(glycerol)}}$$

The remarkable transformation of sugar into the higher fatty acids has thus far not yet been imitated by katalysis, but we can at least obtain lower fatty acids of rancid odour if we mix a diluted *glucose* solution with about half its weight of very active platinum-black ; the odour will be perceptible after several hours and increase gradually. The main action, of course, is a direct oxidation, but simultaneously a reduction is going on in other molecules which yield up a part of their oxygen also to glucose.[1] Platinum-black deprived of its absorbed oxygen in one way or other, is less active in this regard.[2] The rancid smell is not noticed if we boil the mixture of sugar solution and platinum black in presence of some sodium or calcium carbonate, and acidulate afterwards.

Another process, hitherto unexplained, is the easy

(1) *O. Loew*, Ber. Deutsch. Chem. Ges., 23, 865.

(2) Also presence of chlorides interferes very much with its activity. This may be restored by treatment with alkalis and exposure to air after washing.

reduction of nitrates to ammonia in the formation of proteids. As there exists no nascent hydrogen in the living cells, I have long entertained the view that *glucose* under the *katalytic* influence of the *living* protoplasm would be the reducing agent, and have now succeeded in imitating that reduction by means of platinum-black, even at the ordinary temperature. If 50 cc. of a mixture of a five to ten per cent. glucose solution with two per cent. calcium nitrate and 10 g. platinum-black is left to stand for five days in a closed flask, we observe upon supersaturation with potassa a strong ammoniacal odour, while in a control experiment without the platinum no trace is observable.[1] If we modify the experiment by increasing the amount of the nitrate to three times that of the sugar, we find on heating that the acid reaction generated at first, soon diminishes and finally gives way to an alkaline one, and then the ammonia previously formed becomes very perceptible by its odour.[2] The platinum-black imparts to the hydrogen atoms of the glucose molecules motion, thereby loosening the existing affinities, and awakening others; this leads to an exchange of oxygen and hydrogen between the nitrate and the sugar.

In a similar way, chlorates can be reduced to chlorides katalytically; sulphates however resist and evidently require more energy; but at least with a certain sulpho-acid, the combination of form-aldehyde with acid sodium sulphite,

(1) Other experiments prove beyond doubt that only the platinum-black itself and not any oxidation product or bacterial action is the cause of the formation of ammonia.

(2) If the mixture is rendered alkaline *before* the platinum-black is added, no ammonia will be produced, a result which finds its explanation in what has been mentioned above.

I succeeded in effecting reduction; for this, in presence of some sodium carbonate, yields by moderately heating with platinum-black, small quantities of sodium sulphide,[1] while the odour of methyl mercaptan becomes perceptible.

The katalytic action of platinum-black is manifested in still another case of physiological interest. If moistened with pure potassa and exposed to air it forms from nitrogen and water nitrous acid and ammonia to a small extent.[2] Such a process may take place when nitrogen is assimilated by leguminous plants whose roots have entered into symbiosis with certain bacteria. The reverse process can also be katalytically accomplished;[3] for a neutral solution of ammonium nitrite, which is only decomposed by application of *heat*, will in presence of platinum-black show a continuous development of nitrogen at the *ordinary temperature*. A mixture of 6 g. ammonium sulphate with its equivalent quantity of potassium nitrite dissolved in 130 cc. water developed after addition of 20 g. platinum-black in 24 hours 191 cc. nitrogen, and in 5 days as much as 768 cc., at 15° and 723 mm. barometric pressure. A similar process takes place in cases of putrefaction when nitrates are present, where the protoplasm of the bacteria acts upon the ammonium nitrite formed and liberates nitrogen.

To repeat: in all katalytic actions the *platinum-black* remains exactly what it was; it does not act by virtue of any chemical affinities, but only by a specific mode of

(1) We might express this result by the following equation:

$2CH_2OH.SO_3Na + 4Na_2CO_3 = Na_2S + SO_3Na_2 + HCOONa + 5NaHCO_3$

Cf. *O. Loew*, Ber. Deutsch. Chem. Ges., 23, 3125.

(2) *O. Loew*, ibid., 23, 1443.

(3) *O. Loew*, ibid., 3019.

motion. Analogous to this is the action of *living protoplasm*; it remains what it is while it produces various chemical transformations. If it acted *by virtue of chemical affinities*, it would itself undergo a change, and that would mean death, when that change amounted to more than slight traces in the unit of time.

Affinities in the protoplasm, however, come into consideration, when certain compounds become *attached* which thus must yield easily to the katalytic influence, *i.e.*, to the impacts of the protoplasm.

The peculiar energy, then, so well adapted to do chemical work must be a function of the chemical lability of the living matter. We have, in the foregoing chapter, explained this lability as due to a specific atomic motion. This is perpetually being communicated to atoms of those substances that enter into the protoplasm changing thereby the existing affinities in them. The result depends upon different conditions:

1. The intensity which the oscillations of the labile atoms in the living matter have assumed under the influence of the heat of respiration;

2. The specific tectonic of the protoplasm;

3. The degree of chemical resistance offered by the entering compound, and the presence of certain other substances which may readily enter into action under the influence of the living protoplasm.

The power connected with the chemical constitution of the plasma proteids not only plays the part of a liberating energy but also may, supported by heat energy, or by radiant energy, lead to accumulation of potential energy; in other words: *the katalytic actions of living cells may be either exothermic or endothermic.* *Stohmann* (*l.c.*),

however, distinguishes between katalytic and synthetic processes. In the former energy is liberated, in the latter it is absorbed and passes from the kinetic into the potential mode. According to this definition the production of protoplasm from the proteids of the food, or the production of fat, starch, or glycogen from sugar, or that of carbohydrates from carbonic acid in the green plants *would not be katalytic processes proper.* But, I think, this distinction would only complicate the understanding, without any real advantage being gained. Besides, the explanation I have given, is not in contradiction with the laws of *Energy.*

CHAPTER VIII.

THEORY OF RESPIRATION.

THE living organism has frequently been compared with a machine using coal; in both cases a liberation of energy by oxidation and its application for various useful performances are accomplished. This comparison, however, is only admissible within a very narrow compass, since the difference becomes great with an increased supply of oxygen. Then, the coal will burn with much greater vigour, whilst respiration will not be intensified, because the amount of oxygen needed is regulated by the living cell (*Pflüger*).

The respiratory intensity not only exhibits great differences in various species of organisms[1], but also in different organs of one and the same organism.

The intensity is greater in flowers than in leaves and roots,[2] greater in leaves than in stem and fruits (*Saussure*),

(1) To-day it seems hardly credible that *Liebig* still maintained that chlorophyll-bearing plants will not carry on respiration, although *Saussure* had positively proved to the contrary as early as 1805! Another erroneous conception, viz., that the *blood* is the principal seat of respiratory oxidation in animals was corrected in the year 1868 by *Pflüger*, who has recently told us what enormous labour for years it took him to convince physiologists of the truth that oxidations in animals are accomplished by the *cells* of the various organs.

(2) The root-ends of young plants of *Vicia Faba* consume in 24 hours 5 per cent. of their dry matter (*Palladin*). Young rootlets and especially root-hairs have an energetic respiration, while the interior of large roots respires certainly much less than leaves. Not only the restricted access of air but also the lower temperature to which usually roots are exposed at some depth in the soil, will under ordinary conditions lower the intensity of respiration.

greater in light-plants than in shade-plants (*Adolf Mayer*), greater in shoots of oil-seeds than in those of starch-seeds (*Godlewski*), greater in air-plants than in hydrophytes (*Bœhm*), double in the cat what it is in the sheep (*Munck*).

While increase of *pressure* of oxygen will not influence the intensity of respiration, the effect of *rising temperature* is, on the other hand, very considerable, the activity of the protoplasm being thereby greatly enhanced. At 0° the respiration of plants is very slight, at 17–20° it is already twenty times more intense (*Kreusler*), and still gradually increases with the temperature nearly up to that of death. Cold-blooded animals respire less than warm-blooded,[1] while animals in hybernation exhibit a lower temperature and respire less than those in activity.

Heat, visible motion, chemical action, and, in certain cases, electrical phenomena and light are the forms of energy resulting.

Even the finest *currents* in the protoplasm depend upon the presence of oxygen, as *Kühne* has shown, and the increased *work* connected with a rapid development of shoots and embryos, or with the transportation of starch in plants, requires also an increase of respiration. This holds good also for every increase of muscular exertion. The work done by a muscle corresponds to about 33

(1) The intensity in frogs and earthworms is only about one tenth that in the dog; that of higher plants is generally found less than that of warm-blooded animals, in many cases also less than that of cold-blooded, if equal weights of *dry* matter are considered. It is in sprouting peas about one half that of the frog, while in well nourished mould fungi it much surpasses even that of mammalia.—Increase of nitrogenous manure intensifies the respiration of plants (*H. Müller*). Infection with *Peronospora* increases it in the potato plant (*Bœhm*).

per cent. of the energy yielded by respiration (*Zuntz*), while in a steam-engine only 10 per cent. of the caloric value is secured in the form of work.[1]

The function of respiration, however, consists not merely in yielding kinetic energy; it serves also to prepare useful oxidation products, as carbohydrates from fats in plants,[2] and it helps to prepare from different materials the necessary starting groups for proteid formation (cf. Chapter IV).

Besides, not all organisms produce their physiological energy by respiration; the fermentative organisms gain it without the aid of oxygen, by decomposition of organic matter.[3] Bacteria can utilise various hydroxy-acids,

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(1) *Rumford* remarked, long since, that a ton of hay would be administered more economically by feeding a horse with it, and then getting work out of the horse, than by burning it as fuel in an engine.

(2) The formation of organic acids is also due to respiration, viz., to an imperfect oxidation of sugar in most cases. An interesting example is furnished by the flowers of *Ipomæa triloba*, which are *blue* in the morning and remain so during cold, foggy, and rainy days, but turn *red* on warm bright days. Since this change from blue to red can also be easily accomplished by organic acids, we must assume that the increased respiration on warm days causes the production of such acids.

(3) The fact that bacteria can be deprived of their *fermentative* faculties, without their *life* being impaired and thus be turned from anaërobs into obligate aërobs, has led me to the view, that there exists a *special organoid* in those organisms endowed with the function of fermentation. This would, to a certain degree, be analogous to the *chloroplast* of green plants; the latter prepares suitable material for growth from carbonic acid, while the former prepares it by *decomposing* various organic matters. A small fraction of these fermenting compounds does not appear in the form of fermentation products, but in parts of new cells of the fermenting organism. We see, therefore, here also, as in respiration of plant cells, a double part played, viz., that of liberating energy and that of preparing the necessary groups for proteid formation. Cf. *O. Loew*, Centralbl. f. Bacteriol., 9, Nr. 22.

proteids, sugars, and polyvalent alcohols, while the yeasts utilise certain kinds of sugar only. This so-called *intramolecular respiration* yields, *ceteris paribus*, less energy than the normal. Certain animals can, for a limited time, also subsist on intramolecular respiration, as *Pflüger* has observed in frogs, and *Bunge* in worms.[1]

The views propounded by various authors as to the cause of respiration exhibit considerable discrepancy. The oldest hypothesis, that of *Schönbein*, assuming the formation of *ozone*, had soon to be discarded.[2] But nevertheless, the idea that common oxygen had to be changed into an *active form* or modification *before* it could unite with the compounds in the cell, prevailed also in the other theories. The supposed activifying process consisted in the splitting of the oxygen molecule into its two atoms with free affinities. *Hoppe-Seyler* supposed that the living cells produce *hydrogen*, which in its nascent state accomplishes the 'activifying' process by combining with one of the atoms and setting the other free. But it was objected that hydrogen ought to make its appearance at the moment oxygen is being withdrawn. Now, germinating seeds can remain one day, certain worms (*Ascaris*) even 5–7 days, alive in absence of oxygen, but no trace of hydrogen is evolved by these organisms during that time.[3]

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(1) With insufficiency of oxygen, animals will show albumin, glucose, and lactic acid in the urine (*Araki*), also an increase of oxalic acid (*Reale* and *Boeri*).

(2) *Liebig* never discussed the singular fact that carbohydrates and fats are oxidised so easily in the living organism, while they are so indifferent to the common oxygen outside of it.

(3) *M. Traube* contends, moreover, that nascent hydrogen can not activify oxygen; it can merely produce hydrogen peroxide.

Reinke holds that there exist in the living cells easily oxidisable organic matters, '*autoxidisers*,' which are capable of suffering oxidation in contact with molecular oxygen, so as to produce hydrogen peroxide, which under the influence of enzymes brings about powerful oxidations.[1] But *Reinke* omitted to prove that the supposed 'autoxidisers' are really capable of inducing oxidations of *sugar* or *fat*, while, as regards the peroxide, its absence in plant-cells has been proved by *Th. Bokorny*[2] and by *W. Pfeffer*.[3] Neither could it be discovered in animal cells. *Reinke's* view, moreover, could not explain how respiration, throughout such a wide range, is independent of the amount of oxygen present, an objection already raised by *Pfeffer*. But that there exist in many plants easily oxidisable compounds is correct, since many plant-juices acquire soon a reddish or brown coloration, *if exposed to air*. As the 'autoxidisers' of *Reinke*, however, are neither present in *all* plants, nor in animals, this theory cannot be admissible.

The theory of *Traube*[4] assumes the existence of *oxidising enzymes*, which would act as transporters of oxygen, somewhat like nitric oxide in the manufacture of sulphuric acid.[5] The occurrence of such enzymes was not proved

(1) Botan. Zeitg., 1883. Nr. 5 and 6.

(2) Ber. Deutsch. Chem. Ges., 21, 1100 and 1848. *Pringsh.* Jahrb., 17, 317.

(3) Ber. Sächs. Akad. d. Wiss., 1889. The recent assertion of *Bach* was shown by *Cho*, to be again erroneous.

(4) Theorie der Fermentwirkungen, Berlin, 1858. *Virch.* Arch., 21, 386. Ber. D. Chem. Ges., 10, 984.

(5) Analogous processes are the oxidation of aniline by a slight trace of ammonium vanadate (Bull. soc. chim., 45, 309), or the oxidation of nitrogenous compounds when added to a solution of oxide of copper in ammonia exposed to air (*Loew*, Z. Biol., 1878; Journ. f. prakt. Chem., 1878).

by *Traube*, but has recently been demonstrated by *Bertrand* and others.[1] These enzymes are the cause of the colouring of the juices of many plants when easily oxidisable matters are present, as in potatoes, in the roots of *Daucus*, *Beta*, *Lactuca*, and *Taraxacum*. Among the algæ *Zygnema* may be mentioned, whose fresh juice turns black, and among the fungi *Boletus luridus*, whose freshly cut surface turns blue in contact with air.[2] If, however, oxidising enzymes are *present* where easily oxidisable compounds are *wanting*, then the existence of the former can be shown by adding guaiacum solution or hydroquinol, catechol, or pyrogallol. Guaiacum will yield a blue colour, the latter a brown or black one. *Schönbein* observed this blue reaction in various seeds, and especially well in those of *Cunara*;[3] *Molisch* in the secretions of various roots, as those of *Pisum*, *Brassica*, *Cucurbita*, *Lepidium*, *Scorzonera* and *Neottia*.[4]

(1) A year before *Bertrand* published his observations, their existence was positively proved by *Toyanaga* in the laboratory of the College of Agriculture of the Imp. University in Tokyo.

(2) Also the cambial sap of the conifers turns gradually brown in contact with air. The turning brown of blossoms and green leaves which sets in after death, belongs to the same group of phenomena.

(3) Journ. prakt. Chem., 88 and 105; *Gmelin-Kraut*, I. 2. p. 456. *Schönbein* had also observed that these oxidising effects of plant-juices are not met with in presence of ferrous salts or prussic acid. *Pfeffer* had surmised that oxidising enzymes are contained in the cytoplasm, while the oxidisable compounds that give rise to various colorations after death, are accumulated in the vacuole, which explains why these colorations are not noticed as long as the cells are alive.

(4) *E. Schöne* (Z. analyt. Chem., 33, 159) observed that *old* guaiacum tincture yields a greenish blue coloration with diastase alone; *fresh* tincture, however, wants the addition of hydrogen peroxide. The substance yielding the blue product is guaiaconic acid, $C_{20}\ H_{24}\ O_5$ (Lücker).—*Nencki* observed a blue coloration of guaiacum tincture upon addition of benzaldehyde (Journ. prakt. Chem., 26, 25).

I observed, many years ago, that an enzyme-like compound of proteid nature can be precipitated from potato juice by alcohol, which loses its property of blackening hydroquinol upon drying in the exsiccator.

Toyanaga determined the temperature at which the potato lost its peculiar action upon hydroquinol, pyrogallol, and catechol, and found it to be 73°. Mercuric chloride destroyed that property at 55° in one hour, formic aldehyde (5%) after two days standing, almost completely; dilute sulphuric acid and caustic potash acted in the same way, so that there can be hardly any doubt that the active principle is an enzyme.

Oxidising enzymes appear to be present also in the *animal* body. Saliva produces a blue colour with *Wurster's* reagent (tetramethyl-paraphenylene-diamine), a fact erroneously taken to be a proof of the presence of hydrogen peroxide; it produces further a brown coloration with hydroquinol. *E. Salkowski* found in the blood, *Schmiedeberg*, *Jaquet*, and also *Yamagiwa* in the lungs, kidneys, and muscles, enzyme-like agencies, capable of producing small quantities of benzoic acid from benzyl alcohol and of salicylic acid from its aldehyde.[1] That the juice of fresh liver, also pus and milk, colour guaiacum tincture blue and that milk heated to 80° has lost this property, are old observations.

Such oxidations by enzymes are always of a very narrow compass and appear to be limited to certain benzene derivatives; nobody has ever observed that they could attack *sugar* or *fat*, to say nothing of bringing on a complete combustion. The theory of *Traube* has therefore

(1) Jahresb. f. Thierchem., 22, 387. Also *Röhmann* and *Spitzer*, Ber. Chem. Ges., 28, 567.

also to be rejected as being quite insufficient to account for most of the great and varied changes which constitute the phenomena of repiration.

Most of the physiologists of the present day still cling to the idea that oxygen is activified in one way or other. *But such a modification of oxygen as would be capable to completely burn up sugar or fat would certainly kill the protoplasm instead of serving the office of respiration.* We know that the protoplasm is an exceedingly subtle body which is very easily killed by oxidising agencies as *e.g.*, by hydrogen peroxide or potassium permanganate.

It appears singular that just the most important condition for respiration, *i.e.*, the *living state of the protoplasm* has been ignored. Taking a plain chemical start and leaving physiology aside, these physiologists have endeavoured to reach a satisfactory explanation, biased by the idea that the albuminous compounds the chemist studies in his vials are the same as those composing living matter. This erroneous conception still governs the minds of many, and by them respiration will never be comprehended. *Pflüger* was the first who dissented from the general opinion, and declared that "not the oxygen is activified but the proteids of the living protoplasm have the activity."

Respiration exhibits, like the oxidations by various agents,[1] its own specific oxidising action. An animal capable of oxidising in 24 hours several hundred grams of starch (sugar) completely to carbon dioxide and water,[2] is

(1) Potassium permanganate, nitrous acid, hypochlorites, hydrogen peroxide, lead peroxide, silver oxide, have all a specific oxidising action. The results may be modified by relatively small changes in the molecules to be oxidised, as by the introduction of an acidic or alkylic radical.

(2) The question as to the intermediary products is of relatively small importance. The formation of glucuronic acid in animals shows that to a very small extent acids are produced, but formic and oxalic acids are certainly only formed in insignificant quantities.

unable to burn up a few grams of oxalic or formic acid.[1] It is even unable to oxidise 1 g. of benzene completely to phenol. And while it destroys tyrosine, quinaldine, and pyrrol, it attacks with difficulty hydroquinol, phenylacetic acid, or naphthoic acid.

A series of investigations by *Nencki*, *Mering*, *Baumann*, *Salkowski*, and others, led to the recognition that certain compounds reappear in the urine unchanged, such as benzidine; others in combination with sulphuric acid, as phenol; others again (partially oxidised or not) as derivatives of glycocoll, like picoline or benzoic acid; or of glucuronic acid, as tertiary alcohols and thymol; they may also be transformed into uramido-compounds like sulphanilic acid, or taurine. The lateral chains of aromatic ketones are oxidised to carboxyl, if the benzene ring does not contain a hydroxyl-group, otherwise the entire compound will reappear in combination with sulphuric or glucuronic acid in the urine, with the lateral chain preserved (*Nencki*). Some sulphur compounds will yield sulphuric acid, while others will not.[2]

It is, further, of great interest that the oxidation of benzene to phenol and to small quantities of catechol and hydroquinol in the animal body (*Nencki* and *Giacosa*) is diminished by the introduction of certain poisons.[3]

(1) Sodium oxalate in non-lethal doses reappears in the urine with a loss of only 7 per cent (*Gaglio*). Sodium formate reappears to the extent of 50 to 70 per cent. of the dose in the urine (*Gréhant* and *Quinquaud*).

(2) Compounds of the general formula $NH_2—CO—S—R$ easily yield sulphuric acid; thiophene or sulphonal do not (*Smith*).

(3) *Nencki* and *Sieber* (*Pflüg.* Arch., 31, 319). found *e.g.*, that while in the body of a healthy man 0·82 g. phenol was formed from 2 g. benzene, under normal conditions, only 0·33 g. was formed if 2 g. ethyl alcohol per kilo of body-weight were administered at the same time. By various conditions the physiological oxidation in animals may also be *increased*, as *Pflüger*, *Poehl*, *Jacksch* and others have shown.

The difficulty in completely oxidising benzene derivatives of a certain constitution is not only encountered in the animal but also in the *vegetal* organism. Many phænogams accumulate tannins without utilising them again under ordinary conditions; neither do certain alkaloids undergo further metamorphosis.[1] It is, further, very characteristic of the oxidising faculties of plant-cells that certain compounds are left unchanged, which, even by such a comparatively weak oxidising agent as hydrogen peroxide, are attacked readily. Thus, it was observed by *Pfeffer* that cyanine (a quinoline derivative), introduced into plantcells, is not altered, while it is easily bleached by the peroxide. Also certain compounds in the roots of *Vicia* and of *Trianea Bogotensis* are easily converted into brown oxidation products by it, while they remain colourless during their stay in normal cells.[2]

How weak the oxidising power of the cells appears to be here, and then how energetic when sugar comes under action! We search the entire domain of chemistry in vain for a single case of *complete* combustion of an organic compound in *aqueous solution* by the oxygen of the air. However, there exist cases where *partial* oxidations by *molecular oxygen* can easily take place. I may mention the transformation of aldehydes into acids, of hydrazo-benzene to azobenzene, of indigo-white into indigo-blue. Also the behaviour of bibrom-ethylene, anthrahydroquinol, oxindol, and paramidophenol to common oxygen may be cited. Certain compounds become active upon molecular oxygen

(1) Cf. the investigations of *Leo Errera*, *Maistriau*, and *Clautriau*, especially those of the latter in the Bulletin Belge de Microscopie, 1894.

(2) Ber. Sächs. Akad. d. Wiss., 1889, p. 493. These observations likewise proved the absence of H_2O_2 in plant-cells.

through the presence of an alkali, as pyrogallol, pyrogalloquinol, chrysarobin, furoin,[1] while benzene does so in the presence of aluminium chloride. In all these cases it is a *state of lability*[2] that leads to oxidation, a condition that can be either directly connected with the constitution of the compound or can be *imparted*. The latter case has evidently to be assumed when we observe that such relatively stable compounds[3] as sugar, fatty acids, and amido-acids easily undergo combustion in contact with living protoplasm.

It is the CH_2-group in the fatty acids and amido-acids and the CH OH-group in ketoses, aldoses, and hydroxylated acids that are most easily attacked in the living cells. The carboxyl-group renders the CHOH-group in a molecule much more easily attackable than does the alcoholic group CH_2 OH. Thus, we see that glycerol and mannitol are (at least in the animal) by no means easily oxidised, while, *e.g.*, tartaric acid is. The influence of the carboxyl-group also becomes evident when we compare the bibasic phthalic acid with the monobasic benzoic acid; the latter resists while the former is for the greater part burned up.

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(1) A preliminary 'activifying' of oxygen takes place here just as little as in living protoplasm.

(2) Lability of hydrogen linked to carbon may be of two kinds: one which determines its easy exchange by certain metals, as in acetylene; the other which causes its increased affinity for oxygen, as in aldehydes. In regard to the first kind, it may be mentioned that three nitro-groups have (in trinitromethane) the same effect as two carboxylgroups (in malonic ether), (*Claisen*).—A high degree of lability is connected with the hydrogen in diamidogen, which absorbs molecular oxygen with liberation of nitrogen (*Lobry de Bruyn*).

(3) *Nencki* and *Sieber* have tried to determine the extent of oxidation which sugar and albumin can undergo, when exposed at 36° in *alkaline* solution to air, and have found it to be very slight.

That in the animal the *amido-acids* (leucine, glycocoll, tyrosine) undergo combustion with especial facility,[1] yielding thereby urea, was demonstrated by *Nencki* and *Schultzen*, as early as 1872. *Nencki declared, therefore, the amido-compounds to be the forerunners of urea.* This view, which does not assume a *direct oxidation* of dissolved proteids, but a previous splitting into a group of well known amido-acids, is very well supported by observations of *Hofmeister*, which prove with what difficulty peptone, *as such*, is oxidised in the living animal.[2] A rabbit of 1·75 kilo. discharged, after intravenous injection of 0·318 g. peptone, over 80 per cent. of it again in the urine, and after subcutaneous injection about 66 per cent.

The oxidation caused by living protoplasm exhibits evidently great analogy to that instigated by platinum black, which renders alcohol molecules so labile that oxygen is readily taken up with production of aldehyde and acid. This property of platinum black cannot be due to a special chemical activity of the absorbed oxygen,

(1) The amido-acids from proteids are even more quickly oxidised in the cells than the carbohydrates, as must be concluded from investigations by *Kumagawa*, who nourished dogs with an *excess* of lean meat and observed that under this condition the glycogen of the meat was deposited as fat in the animal (Mittheilungen der medic. Facultät Tōkyō. 1894). It is, moreover, of great interest to observe how the production of urea is influenced by chemical constitution: negative groups connected with the nitrogen will prevent the formation of urea, as *Nencki* has shown with acetamide, recalling the resistance of hippuric acid. But taurine and sarkosine are also oxidised with difficulty, reappearing as uramido-compounds in the urine (*Salkowski*).

(2) It may be mentioned that while hydrogen peroxide or platinum black has hardly any oxidising action upon peptone, this is very easily attacked if its solution in ammoniacal solution of copper hydroxide is exposed to air. A moderate amount of oxalic acid is hereby produced (*Loew*, Z. Biol., 1878, p. 294).

since moderately *compressed* oxygen has, at the ordinary temperature, no special chemical action. Nor can the assumption be sustained that platinum black can transform oxygen into an active modification, for we know how easily it transforms, on the contrary, ozone into indifferent oxygen. Oxidations by protoplasm,[1] as well as by platinum-black, have to be looked upon as *katalytic oxidations*,[2] *i.e., as oxidations caused by imparting a specific energy.*

The view here taken as to the cause of respiration, corresponds in its principal feature to the definition *Nägeli* gives of oxidising fermentation.[3] This author says: "the specific state of motion in living protoplasm of the bacteria is extended simultaneously to the alcohol and to the oxygen molecules. If, thus, the equilibrium is disturbed to a certain extent, chemical change takes place by aid of chemical affinities." This theory, however, is still imperfect, as it does not show, how the 'specific state of motion' in the protoplasm is caused, and does not define, whether it consists of a molecular or of an atomic motion.[4] It is the *chemical difference* between the proteids of

(1) How little the *protoplasm* itself in presence of much sugar undergoes the oxidation process may be illustrated by the fact that in special cases 95 per cent. of the matter oxidised may be sugar and only 5 per cent. proteids; in bees the percentage of the latter oxidised is still less.

(2) The old supposition of *Liebig* that the proteids of the food are transformed first into living matter and as such decomposed and yield urea has been revived of late, but it can only to a certain extent be correct.

(3) Theorie der Gärung, p. 43.

(4) *Nägeli* published his "Theorie der Gärung" in the year 1879. After that time I often had discussions with him concerning the difference between living and dead protoplasm, which he had taken for a physical and anatomical one. Later on, however, he agreed that there must exist a *chemical* difference. Nägeli's fermentation theory differs considerably from that of Liebig. Cf. his "Theorie der Gärung" p. 26.

living and those of dead protoplasm which furnishes the key to the 'specific state of motion' which is caused by atoms in labile positions.

As the force of cohesion is counteracted by heat energy, so the atomic cohesion, *i.e.*, the affinities in a molecule, are overcome by plasmic energy. Increased *molecular* motion (heat) can, at a certain temperature, partially pass into *atomic* motion, leading thereby to combustion;[1] plasmic energy however, accomplishes the same result at a much lower temperature, by by driving the atoms asunder against the force of affinity. In other words, the chemical affinities existing between the atoms in molecules of sugar, or fatty acids,[2] or amido-acids constitute a resistance that the charge of kinetic energy derived from living particles can easily overcome, thus inducing the union with oxygen, *normal respiration*.

But if molecular oxygen is absent, then such 'activified' sugar molecules will undergo other changes, with the production of fat, lactic acid, or alcohol; these processes are accompanied by a development of carbon dioxide, and have received the name, *intramolecular respiration*.[3]

(1) A previous activifying of the oxygen is here never noticed, for the small amount of ozone formed by rapid combustion under certain circumstances is only a by-product. The oxygen-molecul is split in the moment of combining.

(2) Fats, *as such*, can hardly serve for respiration, not being soluble in aqueous fluids. It was therefore supposed that a previous *saponification* is necessary. It is, however, much more probable that a conversion of the neutral fats into *lecithin* takes place, which swells up easily in water and is even a little soluble in it. Cf. *O. Loew*, On the physiological functions of phosphoric acid, Biol. C., 11, 270.

(3) The view of several authors that the so-called *intramolecular* respiration is the primary cause of normal respiration has been refuted as erroneous by *Sachs*, *Diakonow*, *God'ewski*, and others.

If the amount of respiratory fuel decreases, the intensity of respiration will diminish also, and, finally, the active proteids of the living protoplasm will, on account of their lability, themselves take up oxygen. And if in a cell a small amount of protoplasm has thus been changed, the equilibrium of the entire tectonic will be disturbed and a rapid chemical change will follow: *death by starvation*. The protoplasm, however, as if endowed with intelligence, understands how to avoid this dangerous result. It absorbs food and instead of itself taking up oxygen, throws it upon the 'activified' molecules of food and *thus derives the greatest profit to itself*, the liberated energy being utilised for various vital functions. A great part assumes the form of heat and is dissipated, another serves to *increase the original plasmic energy* and leads thus to mechanical and chemical performances.[1]

But the increased state of lability will lead in turn to an increased respiration, and this, again, to an increased lability. Thus the temperature would continuously rise also, and another dangerous point would be reached: *death by heat*, at 45-50°C. The increased motions of the labile atoms would facilitate re-aggregation into stable position, and passage into *passive* proteids by the action of the labile groups upon each other; we may define this death, therefore, as caused by an intramolecular self-poisoning. However, there exist, again, conditions to prevent this result, heat being lost by conduction, by radiation, and,

(1) It is but natural that all attempts to explain the amœboid movement, or the locomotion of diatoms and swarm-spores on purely mechanical principles, have thus far, proved failures, since the *chemical* character of the living protoplasm, the energy of lability in the proteids composing it, that forms the fundamental condition of contractility, has been entirely disregarded.

further, by evaporation of water. Higher animals also enjoy the benefit of regulating contrivances in the nervous system. It is, nevertheless, admirable to see how close the temperature of *birds* is kept to that dangerous degree which, like an abyss, separates life from death. And still more remarkable are the few exceptions to the general rule, as observed in certain algæ and bacteria, which can remain alive even at temperatures above 90°C.[1]

If we now consider again the general teaching that "the potential energy of the food yields the kinetic energies of the organism," we must keep in mind that there is originally present kinetic energy in the living matter, which *liberates* that potential energy by combustion, and that it is *the original energy being thus intensified* that leads to the vital functions in the various organs and organisms.[2]

Grant Allen in his admirable treatise 'Force and Energy' gives the following 'Table of kinetic energies':

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(1) Cf. the interesting attempt of *Davenport* and *Castle* to explain these anomalies, Arch. d. Entw.-Mechanik, II, 2. (1895).

(2) There still prevail erroneous conceptions on this point among physiologists, as may be illustrated by a passage of *Pflüger* in his 'Archiv für die gesammte Physiologie,' vol. 10, p. 327: "Die *Wärme ist also die Ursache des Lebens und nicht wie man die Sache gewöhnlich ansieht nur die Folge.*" No doubt, the molar, molecular, and chemical activities of the protoplasm are to be accounted for by the transformation of potential energy of the food, but this is not sufficient to justify that sentence of *Pflüger*, as there must exist a special means—(1) for accomplishing the combustion, and (2) for turning the heat to account. *This* means, as explained above, must also be the first cause of vital activity.

Separative.	Separative molar motion. (In a body raised from the earth's surface).	Separative molecular motion. (In a body torn apart).	Separative atomic motion. (In chemical decomposition).	Separative electrical motion. (In an electrical machine).
Aggregative.	Aggregative molar motion. (In a falling body).	Aggregative molecular motion. (In a body cooling),	Aggregative atomic motion. (In chemical combination).	Aggregative electrical motion. (In lightning).
Continuous.	Continuous molar motion. (In a top or in a planet).	Continuous molecular motion. (Motion in heat).	*Continuous atomic motion. Unknown.*	Continuous electrical motion. (In magnet).

Now, the energy consisting in continuous *atomic* motion, for which *Allen* could not cite an example, must be identical with that displayed by atoms in labile position. The foremost example of such energy is represented by

PLASMIC ENERGY.

CONCLUSION.

It may be convenient to the reader to have the leading facts and inferences recapitulated. Our point of departure has been a theory of proteid-formation in plants, which led to a new conception of the chemical character of the proteids composing living protoplasm, and thence to inferences that proved to be in perfect accordance with observed facts. Waiving all speculation, I can express these *facts* in the following sentences:

1. The transition of living protoplasm into dead protoplasm exhibits a far-reaching resemblance to the transition of a labile substance into an isomeric stable form by atomic migration.

2. Compounds that enter easily into reaction with *aldehydes* are poisonous for all kinds of organisms. Such compounds have no action upon dead protoplasm or upon common proteids.

3. Compounds that enter easily into reaction with *labile amido-groups* are poisonous for all kinds of organisms, but these compounds have an action upon ordinary proteids also, and, therefore, also upon dead protoplasm (cf. Chap. VI).

4. There exists, widespread in the vegetal kingdom, a highly labile proteid serving as reserve material, which undergoes chemical change by the same influences as those which cause the death of the cells (cf. Chap. V).

The evidence to be drawn from the information supplied by nature leads to the very same conclusions as those I had already reached by deduction. They are (1) that a great activity, in the form of oscillations of certain atoms in labile position, exists in the proteids of living protoplasm, and (2) that this ever active chemical energy, leading to respiration and in turn intensified by it, is especially well adapted to do chemical work, since atoms can be set in motion by others already in motion, just as molecular motion (heat) can be imparted to other molecules, *i.e.*, conveyed by impact. As I fully agree with *Huxley*[1] when he says: "it is a favourite popular delusion that the scientific inquirer is under a sort of moral obligation to abstain from going beyond the generalisation of observed facts," I venture to offend against that popular delusion and conclude that the *peculiar mode of motion* in the *labile proteids* is also the source of *vital activity*. This *Energy* is the necessary link in the chain of constructive and destructive metabolism. It must, on the one hand, help radiant energy to construct carbohydrates from carbonic acid in green plants and, on the other, lend its aid to burn up carbohydrates, fats, and amido-acids in the respiration process.

However, not only the *potential* energy of the thermogenes, but also the *kinetic* energy of the labile proteids composing the living protoplasm, is, in the long run, but one of the vicissitudes of Solar Energy.

I have not failed to point out that the expression 'living molecules,' used by some authors, is not justified, since every life-action is the result of the working of a complex machinery. Even the simplest function of a living cell

(1) The advance of science in the last half-century.

depends upon the proper working of an *apparatus*, which, in some cases, may be represented by a mere fibre, but which must, even then, be built up of an immense number of molecules. *Living particles* exist, *living molecules* do not. To avoid any misunderstanding, I have proposed the name *active* molecules to designate the condition of the molecules in living matter, and to indicate a distinction between them and other labile proteids, such as enzymes and toxalbumins.

What I have attempted has not been an explanation of complicated vital *functions*, but merely that of the nature of the *Energy* emanating from the proteids of living protoplasm. The reader will judge whether the evidence which has been adduced suffices to substantiate the main proposition with which I set out.


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